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A TRANSLATION OF BECCARI'S LECTURE "CONCERNING GRAIN" (1728) [1]

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In 1728 an Italian scientist, Beccari, delivered a lecture before the Academy at Bologna in which he described his experiments involving the separation of gluten and starch from wheat flour. It was not until 17 years later that his observations were published, however, and then apparently not by Beccari himself as author, but by some anonymous commentator or editor. This appears to be the earliest record of investigations of gluten. The journal in which this article appeared is not found in many American libraries, and one may suspect that many authors who have cited it during the past half century have neither seen nor translated it. Printed in medieval Latin, the original would be available only to students of that language, even though they had access to the document itself.

Because of its unique position in the literature of flour chemistry, and in view of its unavailability to many Americans, it was decided that a complete translation should be made and published. This was done by Mr. Loenholdt, who prepared initially a very literal translation. Then I undertook to revise and alter this first effort, to conform somewhat more closely to modern terminology, without sacrificing too much of the style of the original author. This will account for the somewhat flowery style of the translation in certain sections on the one hand, a style quite different from that to which readers of this journal are accustomed, and also for the departure at times from a precise translation in the interest of smoothing and rendering somewhat more understandable the eighteenth century type of expression. Thus a critical Latin scholar could find numerous departures from an exact translation on comparing my version with the original, but where that occurs it has been with the de-

[1] Miscellaneous Journal Series Paper No. 444, Minnesota Agricultural Experiment Station.

sire and in the attempt to portray more clearly Beccari's procedures and the bases for his reasoning. This has necessitated in numerous instances a change in the punctuation.

It is interesting to note the persistence, even in the relatively modern period in which Beccari's work was done, of the ancient effort to classify materials as animal, vegetable, or mineral. This tendency did not terminate in the eighteenth century either, but appeared again when, over a century later, Liebig attempted to describe the individual gluten proteins under such terms as "plant gelatin" and "plant fibrin," while Ritthausen referred to "gluten casein."

So far as I can discern, this announcement of Beccari's studies marked the close of active investigations in this field for over eighty years, at least so far as is disclosed by publications. It wasn't until 1810 that the next major contribution appeared in the researches of Einhof, who fractionated gluten itself by using alcohol as a solvent. Probably the resurgence of activity during those early decades of the nineteenth century was the result, in part, of improved facilities and techniques, including more reagents than were available during Beccari's time. Beccari worked during the period when physical science was still bound by the traditions of alchemy, at least in the matter of techniques; Liebig and his contemporaries found themselves caught up in the chemical renaissance that resulted from the organic chemical interests and discoveries of their time.

But that is another story; I started out in the attempt to fit Beccari's important investigations into the setting in which he found himself, and, measured by the magnitude of the facilities available to him, he made a major contribution with which students of this subject may well be familiar. It is my hope that additional translations of some of the other classical papers may be published from time to time to make them more conveniently available to those who are interested in, and can make use of, the works of the pioneers. Some of those scholars had more knowledge of phenomena and materials than we "moderns" are prone to credit them with, until we read their publications carefully and critically.

CONCERNING GRAIN

(The announcement of Beccari's lecture before the Academy of Bologna, translated by F. Loenholdt and C. H. Bailey, University of Minnesota. Translated from "De frumento" in *De Bononiensi Scientiarum et Artium Instituto Atque Academia*, Volume 2, Part 1, pages 122–127 (1745).)

In regard to the virtue of food, there are those who recognize it and those who fail to do so; verily those (who do) may appear, like the oracle of Apollo, to pronounce in anticipation so that it may be known by all.

Now, if we are to investigate so worthy a subject, may we receive the immortal and indeed divine motivation, by which alone we are, and indeed by which alone we are nourished? Very rightly, the Greeks, the Romans, the Arabs and all those who *summa cum lauda* are following them, the Gauls and the Italians put forth their efforts to determine and explain the nature of foods. Their worthy studies stand out so that we may well admire their excellent observations—indeed we may wonder what they did not observe. It is splendid indeed to investigate the matter of foods and to derive hope from the extent and the almost infinite variety of such materials.

In realizing this, Beccari did not hesitate to apply his diligence to foods which may be of the greatest value to a community and of which all are partakers. Thus faced, he discovered two species of substances in wheat flour, strangely different in themselves, which can be separated with ease, and be discerned as individuals; on the one hand, one may disclose the similarity of these things which are extractable from vegetable bodies, but in this there would be nothing worthy of admiration; on the other hand one may seek and compare, as is possible with those materials removed from the bodies of any living creature; this Beccari reported very admirably; it may well be wondered as to how other authors failed to record similar observations, but nowhere is this evident. He communicated this study to the academy in 1728 in a long lecture to which detailed reference will be made.

First, however, the two parts of the flour to which reference has been made will be explained. They may be obtained with little difficulty. Flour is obtained from the best wheat, moderately ground so that the bran will not pass through the sieve; from this it follows, therefore, that the product is of the cleanest with impurities removed. This is mixed with the purest water, and is kneaded. The residue obtained in this operation is accomplished by washing. The water, therefore, carries away all the portions that can be dissolved; the other portions it leaves behind intact.

Moreover, those portions which the water leaves behind, manipulated by hand and pressed under the residual water, are little by little gathered in a soft and—as much as possible—a tough mass; an excellent kind of paste, and most suitable for the work that is to be done; it is most noteworthy how it does not permit itself to be mixed with water. Those other portions, which the water carries along with itself, remain suspended a considerable time and render the water milky; afterwards they settle downward little by little and collect at the bottom, yet they do not cohere very much with each other; at the slightest disturbance they again float upwards like dust, as it were. Nothing is similar to this starch ("amylo"); this food is truly amylaceous. But it is in fact of two

kinds of parts as Beccari proposed on the basis of his chemical work, and to which he applied names to distinguish them, calling the one glue-like ("glutinosum") and the other starch-like ("amylaceum").

So great is the difference of these two fractions that if subjected to resolution by digestion, or by distillation, one obtains principles from each that are quite different; the starchy material yields principles which disclose its vegetable origin, whereas the gluten when thus treated behaves as though of animal origin. So that this may be understood better, it may be convenient to know in general just how they exhibit diverse characteristics which are as of vegetable and animal nature, and are disclosed in digestion, and in distillation.

In digestion, which is effected by low heat of long duration, the parts of any animal are never brought to a real and absolute fermentation, but they are always caused to putrefy with a very bad odor. The vegetable parts may be fermented naturally, as it were, and not caused to putrefy unless this is brought about artificially; they yield certain characteristics of sourness during fermentation which are in no way like the behavior of animal parts when they are caused to putrefy. Also, after long continued fermentation, the vegetable portions may produce an acetic liquor which has a savour of wine; the animal portions, when caused to putrefy, yield a urine-like liquid. Out of one a strong spirit can always be recovered, and appreciable acid salt; out of the other (animal) there is no trace of acid salt but not a little alkali. Finally, the vegetable portions, when they have been fermented, relinquish a substantial amount of *tartar* and of stable salt; of which salt none appears when animals are caused to putrefy. These are the differences between vegetable parts and the parts of animals in digestion.

Whereas the fresh and sound parts of animals and plants may be so distilled, they differ also when dried over a strong fire. From the animal parts water is first extracted, entirely devoid of taste; then a vapor becoming yellow and also alkaline; abundant salt follows this, equally alkaline, volatile, solid, and blended in a yellow oil, indeed beautifully golden and rank; if the salts become separated from the oil by the heat, they become bright like snow. In an extremely increased fire another heavy oil appears, dark, viscous, with a very bad odor, adhering to the bottom of the vessel, sponge-like, flat in taste, dark, and unlike anything above mentioned; later this can be whitened on the open fire as has been shown. However much one washes this out, one may extract no salt. Such is the distillation of animal parts. The vegetable parts, however, produce first a watery liquid, very acid, which becomes more acid over an increased fire, and also more reddish; meanwhile a light and yellow oil comes out; finally, at extreme heat the vapor becomes very acid and thick; likewise the heavy, thick and dark oil floats like a fish.

In this way the plant and animal parts differ, as to chemical considerations, as we have summarized; so they are seen to produce alkaline principles elicited from animal parts, and acid principles from vegetable parts.

After this discussion concerning them, to which we have digressed, we again return to the parts of wheat flour, starch and gluten; as they emerge from any one substance they are discernible as such, and they may be had for demonstration either in digestion or in distillation; for starch, as I have said above, appears to be entirely of vegetable nature; gluten, however, exhibits the characteristics of animal parts, so that whoever may not know it to have been taken from grain might assert without the slightest doubt that it came from animal material.

We may begin with digestion. This glutenous material produces no characteristics of sourness; it smells bad within a few days and spoils; it putrefies strikingly like a dead body; it rots, it may become fluid, and it may then be dispersed in water, leaving very little blackish material in the container; it produces quickly a mass like a spoiled carcass. Starch, however, does not initially yield an acid, then sours perceptibly, and finally contracts the odor and flavor of wine and becomes blended with the supernatant liquid. The first (gluten) is characterized by its tendency to rot, the latter (starch) by becoming sour; surely Beccari might have obtained this less in a temperate place, since, for instance, in the more than forty day period during the warmest time of spring spoilage would have occurred more easily, nevertheless he preserved the odor and somewhat acid taste, always fresh and unspoiled.

He preferred to conduct his experiments in water, in which the digestion of either the glutenous or the starchy part may be achieved. In the instance of the starchy part, all comes to the same state; indeed, the water comports itself as though it was accustomed to blending with the acids that are produced. Nothing is changed during the mixing of the other acids; with the alkaline salts, as for instance with the tartar salts, it effervesced slightly indeed but sufficient to foam over, and, furthermore, lost its clearness. Then it threw off whitish particles, followed by a violet color which merged into a red color that was retained.

The water which was affected by the glutenous portion, however, acted in a manner as though the alkaline juices are accustomed to it. It effervesced, due to the mixing of its acid; it changed in color, and, after a few days it settled to the bottom as a very fine powder.

Beccari was unwilling to remain silent concerning these particulars, which have been known with certainty in these color changes which are mentioned above. These he narrated, therefore. The liquor extracted an unfading color out of the water in consequence of strong mixing, of beautiful appearance which becomes visible after more or less saturation.

The liquor becomes a reddish color, with a constant acetic content. If Ettmullerus had actually recognized this when he tried to discover the change in color in chyle, the experiments of Fluddius might not have been made. Fluddius elicited such a substance out of bread, which was very white, but when carefully closed up in a transparent vessel and placed in the sunlight, developed little by little either a purple or red color much as does chyle which may be very white and change into blood-red. As Beccari assumed, the change of color which he observed may be attributed to the same phenomenon.

We now return to the proposed " liquid." When disturbed by added water which has imbibed mercuric sublimate it becomes turbid; with water which has imbibed salt of lead it is no less turbid; with alkali it does likewise. Moreover, it deposited a very fine and white powder at the bottom which, most certainly, is produced by the volatile alkali. Thus both fluids from the respective portions from which they were obtained, may be clearly divided naturally; further, so far as may be manifested by the distillate, the moisture which is expressed by distillation out of the latter (starch) had all the characteristics of an acid nature, and that out of the gluten, the characteristics of an alkaline nature.

While these characteristics may be discovered by digestion of these two portions of wheat flour, that is, the gluten and the starch, the distillation did not disagree with the digestion. Also when these materials, heretofore thoroughly discussed, are not brought to a state of decay by digestion, they may be experimented with by distillation; the gluten portion which yields little or a small amount of water, after a little gives off a vapor similar to urine, followed by an oily material, and then some rather strong, volatile salt such as is given off by such treatment of a stag's horn. The starchy part indeed, yielded nothing at first except a little water, then much vapor of acid, and last of all not a little oil; always, however, it yielded characteristics of an acid nature which attest to its vegetable origin.

Thus not only the chemical digestion but also the distillation has shown the two natures, and these in themselves very different, in one and the same flour. The investigation of this thing allured Beccari. For this reason he desired to prove the same also in flour from beans, from barley, and other things. But there was no like response except in the case of spelt. With the other things the water rinsed everything away so that no glutenous or other compact material remained behind which could be compared with the gluten of wheat; so that Beccari marveled at so great an unlikeness in similar things. All of these flours, as in the instance of the starchy material to which reference has been made, when diluted with water, and properly heated at the right temperature, came together in a cohesive mass, so that they may be used

as a glue on paper; but this has nothing to exhibit in common with the paste of flour. The starch of wheat, if and when opportune, acquired a firmness, and this firmness alone is better than in all flours; also of barley, which attracted the first admiration of Beccari, so that he formerly supposed this flour to be the most cohesive of all. He confirmed this experimental opinion. The old investigators had observed this and maintained that foods made from barley would give acute trouble to hard workers, and bread which is made from wheat they called the best of all. However this may have been, it certainly is evident that Beccari discovered this advantageous unlikeness in many kinds of flours, and, what is still greater, the differences in the constituents of wheat flour alone; these, however, are less significant if we recognize how varied and multiple are the interrelations of all things. However, it was the custom of the scientists of the past that they strived to obtain a likeness and a constancy, but due to their nature, they may have been misled as to the variability. Also it is not apparent that this variability may not have been more beautiful than the perpetuity which they sought.

This statement concerning grain and flour has been presented so that we may interest others, if possible, to follow Beccari in the study of the materials in foods; it is indeed a study worthy of a scientist in which the matter of being ill as well as of being healthy may be investigated.

BIOLOGICAL ELIMINATION OF GLUTATHIONE FROM WHEAT GERM AND FLOURS USED IN BREAD MAKING

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The injurious effect of wheat germ on dough and loaf properties is a fact that has been established for a long time. Different explanations for this phenomenon have been brought forward. Lipoids (Working, 1924; Sullivan and Near, 1928; Geddes and Larmour, 1933), proteases (Cairns and Bailey, 1928; Balls and Hale, 1936a, 1936b, 1938; Jørgensen, 1936, 1939; Flohil, 1936; Hale, 1939), fatty acids (Swanson, 1934; Walde and Mangels, 1930; Kosmin, 1934a, 1934b, 1935; Barton-Wright, 1938), and reduced glutathione (Balls and Hale, 1936a, 1936b; Jørgensen, 1936; Sullivan, Howe, and Schmalz, 1936, 1937; Ford and Maiden, 1938) have in turn been made responsible for the harm done by germ and germ-containing mill streams to bread texture. The last-mentioned

explanation appears to be the one that is best supported by facts. It seems, moreover, to account for the favorable effect of potassium bromate and other oxidizing bread improvers, and for the improvement of flour by heat.

In the course of a study of the effects observed in ripening doughs, one of us (E. W. H.) set experiments to see whether the "greening" effect of the germ could be eliminated by a process of prefermentation with yeast. It was found that when a ferment consisting of wheat germ, water, and yeast was allowed to ferment for a number of hours and was then made into a dough, the bread made therefrom no longer showed the harmful effects of germ on baking quality. Later it was found that at the point at which good bread could be made from the ferment, reduced glutathione could no longer be detected with the sodium nitroprusside reaction.

From these observations a commercial process was devised which allows the addition to bread of up to 10% of wheat germ (on a flour basis). There has already been published (Hullett, 1940) a brief note on this process, in which was described also the improvement resulting from prefermenting the low-grade portions of very high extraction flours and in which it was suggested that glutathione elimination by yeast might be a part of the ordinary dough ripening process.

The experiments and findings made in working out the process outlined above are thought to be of sufficient general interest to justify their being published, although they raise problems rather than solve them.

The flour and germ used in the experiments were supplied by various New Zealand mills and were milled from New Zealand and Australian wheat. Throughout the present investigation the germ was used in uncomminuted state. The presence of glutathione in the germ was checked by means of the sodium nitroprusside reaction. For the test a sample of the germ suspension or paste was diluted to a proportion of 1 part germ to 30 parts water and strained through bolting silk. The glutathione used was a product from Hoffman-La Roche, Basle.

Effects of Glutathione and of Germ on Dough

The results obtained by earlier investigators are fully confirmed by the farinograms and the results of the baking tests carried out by the authors. It was found that the injurious effect of germ additions was greater with some flours than with others. According to Jørgensen's theory this would imply variation in the protease content of the flours.

Chemical changes due to an addition of raw or of fermented germ to the dough seem mainly to consist in an increase or decrease of cold water extract, which can be seen from Table I.

TABLE I

COLD WATER EXTRACT

Additions on flour basis: 2% yeast; 2% salt; 1% cane sugar; 4% raw germ or 12% moist germ ferment equal to 4% germ. 4¼ hours' fermentation at 82°F. Two knocks after 2 and 3 hours.

	Fresh dough	Fermented dough	Bread
	%	%	%
No germ	4.9	4.2	5.0
Raw germ	5.5	5.0	5.4
Fermented germ	5.0	5.0	4.9

Considering that a content of 5% cold water extract in the dough means that the liquid phase surrounding the gluten strands has a concentration of roughly 11%, it is possible that changes in its composition might affect the gluten properties, and from this viewpoint the increase in cold water extract caused by the addition of raw germ should be noted (cf. Kosmin, 1933). But the evidence is insufficient to explain the effect of germ in terms of chemical composition. More detailed chemical analyses were carried out but up to the present no clear conclusion has been reached.

Prefermentation of Germ and Dough Properties

Table II gives observations on a ferment sufficient for a sack of flour (200 lbs). Enough of the ferment to make one loaf was taken at the sixth and following hours, and doughed up at 73°F. Each dough was allowed to lie for half an hour, rounded up, left for half an hour, and then tinned up. All the loaves were satisfactory from the seventh hour onwards. The crumb color varied from a light brown in the sixth hour to a pinkish brown in the 11th hour. This is discussed later.

TABLE II

PREFERMENTATION CONDITIONS SUITABLE FOR 200 LBS. FLOUR

Formula of ferment: germ, 20 lbs.; yeast, 1 lb. 7 ozs.; water, 3½ gals. The "gassing power" is the cc. of gas given off by 78 g. of the main ferment enclosed in a jar embedded in the main ferment so as to experience the same temperature changes.

	Hours								
	1	2	3	4	5	6	7	8	9
Gassing power for each hour (*cc.*)	95	150	173	216	186	120	81	35	20
Ferment temperature (*degrees F.*)	74	74	77	80½	82	82	80	78	76
Glutathione test: + = present − = absent	+	+	+	+	+	slight	—	—	—

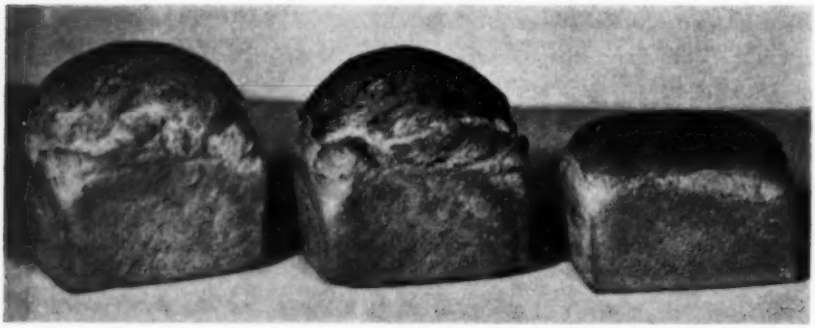
It was apparent that once the glutathione had disappeared, all the loaves were satisfactory, which shows that, if necessary, a baker could leave the ferment beyond the time it is ready and still get good bread. It is a striking feature of the addition of fermented germ to bread that it has a maturing effect and gives good tolerance to the dough. Doughs containing 10% (on flour basis) of fermented germ were found to yield fully satisfactory loaves after two hours' fermentation (from mixer to oven) in the dough, and to continue to give equally satisfactory ones over a period of six hours. This at least applies to New Zealand flours; it may be different with other types of flours.

The following examples show how the process is carried out commercially:

(*a*) 20 lbs. of germ, 6 lbs. of yeast, and 4 gallons of water are made into a ferment, which is kept at 80°F. for 4 hours. 200 lbs. of flour, 4 lbs. of salt, any other dough ingredients, and the required water are mixed to a dough, from which loaves can be taken off starting from 2 hours after mixing.

(*b*) 20 lbs. of germ, 1½ lbs. of yeast, 4 gallons of water, fermented at 74°F. for 9 hours. Dough making as under (*a*).

As has been mentioned above, the practical use of the process is not limited to wheat germ additions only. It has been found that the principle of prefermentation can successfully be applied in improving the baking quality of low-grade flours. A very satisfactory loaf is obtained when low-grade flour (the fraction 70%–80% on wheat basis) is prefermented and straight flour (0%–70% extraction fraction) is added in dough making. The photographs (Fig. 1) show a normal bread loaf,



Normal loaf 10% fermented germ 10% untreated germ

Fig. 1. Effect of prefermentation with 10% of wheat germ in the flour.

one containing 10% (on flour basis) of prefermented germ, and one containing 10% of untreated germ.

If the branny part of a whole meal is prefermented separatedly, and the remainder added when dough is made, an improvement over an ordinary straight whole-meal dough is obtained with wheats of poor baking

performance. Rat-feeding trials [1] showed that the vitamin B_1 of the wheat germ was not destroyed by the process.

It might be argued that the maturing effect is due to yeast multiplication taking place during the fermentation of the wheat germ. In order to make sure whether this was the case, yeast cells in fermented and unfermented germ ferments were counted, with the following result:

20 g. germ + 40 cc. suspension of 10 g. yeast:
(*a*) kept in refrigerator for 4 hours—1,040,000 cells per mm.^{3}
(*b*) kept at 82°F. for 4 hours—1,120,000 cells per mm.^{3}

The yeast cells were roughly separated from the ferment by the method which will be described later under the heading "Effect of Germ Fermentation on the Yeast." The difference of 80,000 cells per mm.^{3} falls within the margin of error of the counting method. Hence the maturing effect is not due to yeast multiplication.

Characteristics of Germ Fermentation

The fermentation which leads to the destruction of glutathione in the germ is connected with an enzyme mechanism effective in the raw germ. This is evidenced by the following:

1. Glutathione is not destroyed by boiling of germ, and when boiled germ is fermented with yeast, there is no destruction of glutathione as there is with raw germ. The boiling evidently destroys the enzyme.

2. Fermented raw germ contains no glutathione SH but may be supposed still to contain the enzyme and therefore to have the power of bringing about the elimination of the glutathione present in a ferment of boiled germ. This does in fact take place.

3. Pure glutathione was added to (*a*) a fermenting sugar solution, (*b*) a ferment of boiled germ, and (*c*) a ferment of unboiled germ. Only in the last case was the glutathione destroyed.

Study of the Rate of Glutathione Destruction in Fermenting Wheat Germ

Some indication has already been given of the speed of the process. It was found that the time taken for the destruction of glutathione could be considerably shortened by increasing the yeast quantity and lengthened by decreasing it. For instance, the time required for glutathione destruction with the same initial conditions as given in Table II but with four times the yeast quantity was 3 hours to 4 hours according to the glutathione content of the germ.

With commercial formulas employing relatively high concentrations of the germ and yeast, temperature variations are very difficult to avoid.

[1] Kindly made by Dr. Muriel Bell of the Nutritional Research Department of the New Zealand Medical Research Council.

It was therefore realized that a more dilute system was better suited to a study of the effects of variations in the concentrations of the different constituents. Tables III, IV, and V record observations on relatively dilute ferments.

TABLE III

EFFECT OF GERM AND YEAST CONCENTRATION ON GLUTATHIONE DESTRUCTION IN GERM SUSPENSIONS CONTAINING 75 CC. OF WATER, FERMENTED AT 82°F.

Wheat germ	Yeast	SH reaction
g.	g.	
2.5	1.0	− after 5 hrs.
2.5	2.5	− " 4½ "
2.5	5.0	− " 4–4½ "
5.0	1.0	− after 6½ hrs.
5.0	2.5	− " 5½ "
5.0	5.0	− " 5 "
10.0	1.0	+ after 6½ hrs.
10.0	2.5	± " 6½ "
10.0	5.0	$\underline{\pm}$ " 6½ "

− means negative; ± weak positive; $\underline{\pm}$ almost negative

TABLE IV

EFFECT OF pH ON GLUTATHIONE DESTRUCTION IN GERM SUSPENSIONS FERMENTING AT 82°F.

20 g. germ + 10 g. yeast in 200 cc. diluted HCl. Yeast: liquid

Initial pH	Glutathione reaction after 5 hours
4.45	+
4.72	±
5.12	$\underline{\pm}$
5.58	$\underline{\pm}$
6.10	±
6.41	+

TABLE V

EFFECT OF TEMPERATURE ON GLUTATHIONE DESTRUCTION IN GERM SUSPENSIONS FERMENTING AT DIFFERENT TEMPERATURES

20 g. germ + 10 g. yeast in 100 cc. water

Initial temperature	Time required for glutathione to disappear
82°F.	4 hrs.
94°F.	3 "

The rate of glutathione destruction in fermenting unboiled germ can be supposed to depend on the following factors: concentration of glutathione, concentration of the active enzyme, and probably rate of fermentation. Temperature and pH, besides affecting the rate of fermen-

tation, might have a specific effect on the enzyme. It was not possible to study the effect of separately varying the glutathione and enzyme concentration, as these two are associated in the germ and no adequate supply of pure glutathione was available.

Glutathione destruction was slightly accelerated by using a 0.002*N* solution of sodium cyanide instead of water. When a 0.001*N* solution of iodoacetic acid was used instead of water, gas production as well as glutathione destruction was entirely stopped. Yeast treated with acetone failed to produce fermentation and no glutathione destruction took place. Neither did glutathione disappear when sodium fluoride was applied to such an extent as to stop fermentation. Thus it was not found possible to disconnect the destruction of glutathione from fermentation.

The conclusions from these trials can be summarized as follows: Increase of temperature (up to about 35°C.) and yeast quantity and decrease of initial pH to 5–5.5 shortened the time required for the SH reaction to become negative. The figures in Table III appear, besides, to indicate that decrease in germ concentration accelerates glutathione destruction. But the converse is found with the tight ferments used commercially. The impression was received that this is due to opposing effects of glutathione and enzyme concentration in the germ.

A series of gassing tests was run in order to find out whether the fermentation of raw germ under different conditions exhibits, in the gassing rates and total gas production, any characteristics differentiating it from the fermentation of boiled germ suspensions or of sugar solutions of comparable sugar and yeast concentrations. The use of a 0.001*N* solution of iodoacetic acid instead of water in dough making produces slackening and stickiness of the dough. Farinograms obtained from such doughs indicate a strong degrading effect taking place.

In order to obviate sugar production by amylases acting on any starch in the germ samples the experiments were carried out on centrifuged extract. The germ used contained 0.5% reducing sugar (expressed as invert sugar) and 14.7% of sucrose, making a total of 15.2%. The sugar content of the sugar solutions without germ was adjusted accordingly.

Germ extracts of two concentrations and solutions with corresponding sugar concentrations (1.5% and 1%) were fermented with an addition of 5 g. of yeast per 100 cc. The initial gassing rate of the germ extracts was higher, no doubt owing to their high protein content and the presence of vitamin B_1 (Schultz, Atkin, and Frey, 1937). The totals for four hours' fermentation and the shape of the graphs of gassing rates give, however, no indication that the fermentation going on in fermenting raw germ is of a type different from that taking place in fermenting boiled germ or in fermenting sugar solution.

The decrease of pH taking place during fermentation was also practically the same with raw and boiled germ, *i.e.*, from about 6.7 to about 5.5.

Color Formation in Fermented Germ

In all our experiments, whatever concentration of germ or yeast was applied, the surface of the fermented suspension or paste began to turn dark brown, later violet, as soon as the glutathione had disappeared and this color deepened as time went on. The bread likewise acquires a color varying from almost white to a pinkish brown, depending on the period of fermentation after glutathione has disappeared. This process seems to be due to oxidation of carotene by air. This is concluded from the fact that extraction of carotene from the germ by means of petroleum ether prevents the color from developing. Low temperature fails to prevent the formation of the color on the surface of fermented germ suspensions, but absence of air or substitution of air by CO_2 does prevent it. Addition of sugar to the ferment also retards the development of the color, and this is no doubt due to sustained CO_2 production.

When the initial pH of the germ suspension is adjusted to values from 5.5 downwards a very noticeable repression of color formation takes place. Bread baked with an addition of acidified germ ferment (equalling 10% germ on flour basis) was of almost normal color. The pH adjustment was made by means of HCl (1 cc. 0.10*N* HCl for every gram of germ).

No color formation occurs in boiled ferments, which suggests that an enzyme mechanism is involved in this process also.

Fate of Glutathione in Germ Fermentation

It has been suggested by different authors that glutathione undergoes oxidation when germ is allowed to age or is heated under certain conditions. These authors also attributed the maturing effect of $KBrO_3$ to glutathione oxidation. Starting from this hypothesis we originally anticipated that the disappearance of the glutathione reaction in the course of fermentation was caused by oxidation to the SS form. In order to test this hypothesis attempts were made to reduce the supposedly oxidized glutathione. Reduction was attempted by: (*a*) saturating the solution with H_2S in presence of a few drops of mercury; (*b*) using aluminum amalgam in neutral solution; (*c*) using zinc and sulfuric acid; and (*d*) using sodium cyanide solution. None of these methods yielded the least trace of reduced glutathione.

In order to make sure that the reduction of the S–S form to the SH form could indeed be effected by these methods, pure glutathione (SH)

was oxidized according to Hopkins (1929) and the solution treated with zinc and sulfuric acid. A strong reaction with sodium nitroprusside was obtained after two hours, and this increased with longer reduction. (In the course of these experiments it was noticed that glutathione is easily adsorbed by various precipitates. In using aluminum amalgam, for instance, the glutathione SH is fixed to the aluminum hydroxide formed in the course of the reaction. The use of zinc has the advantage that the red color obtained with sodium nitroprusside and ammonia is much more stable in the presence of zinc hydroxide than otherwise.) The precipitate formed by the addition of sulfosalicylic acid (used by Sullivan, Howe, and Schmalz, 1937) was also found to adsorb glutathione.

As it seemed possible that the SH reaction of a germ extract might be masked by substances occurring in the extract, the following experiment was made in order to test this point: 0.025 g. of pure reduced glutathione, dissolved in 15 cc. water, was oxidized by adjusting the pH to 7.6 with $Ba(OH)_2$ and aerating until the sodium nitroprusside reaction became negative (Hopkins, 1929). This oxidation required six hours. The solution of the oxidized form was added to 30 g. of germ which had been fermented until the SH reaction had become negative. After thorough mixing, the suspension was centrifuged and the liquid treated with zinc and sulfuric acid. On standing overnight the solution gave a very strong SH reaction, which demonstrated that the nitroprusside reaction is not interfered with by any constituent of the fermented germ. It was likewise found impossible to obtain a positive SH reaction by reducing an extract from germ which, after having been kept in a closed container for several months, did not react any more with sodium nitroprusside. It can be inferred from this evidence that in fermenting or in aging germ the glutathione is destroyed rather than oxidized.

On the strength of the above-discussed findings it may seem doubtful whether the action of potassium bromate in a dough is indeed an oxidizing one. The view that $KBrO_3$ does not cause glutathione oxidation, at least not under the conditions prevailing in baking, is further supported by the results published by Ziegler (1940), who found that below 40°C. the oxidation of glutathione by $KBrO_3$, particularly in a CO_2-saturated solution, proceeds very slowly.

The practical aim of the authors' experiments did not justify an extensive investigation of what actually happens to the glutathione under the conditions discussed above. So much only was ascertained, that the destruction is not connected with H_2S development, as no blackening of lead acetate by the vapors coming from fermented germ could be detected.

Effect of Germ Fermentation on the Yeast

Attempts to find out whether the disappearance of glutathione from the germ extract corresponds to an increase of glutathione in the yeast cells led to the discovery that, on the contrary, the glutathione content of the yeast is greatly diminished. This is shown by the following experiments, in which a comparison was made of the glutathione contents of (*a*) yeast which had been fermented in a raw germ suspension, (*b*) yeast which had been mixed with a raw germ suspension which was kept in the refrigerator, and (*c*) yeast which had fermented a sugar solution of equal sugar concentration.

(*a*) 20 g. germ plus a 40 cc. suspension of 10 g. yeast in water, fermented at 32.2°C. for 3½ hours—SH reaction of fermented germ was negative.
(*b*) 20 g. germ plus a 40 cc. suspension of 10 g. yeast, kept in the refrigerator for 3½ hours—SH reaction positive.
(*c*) 3 g. sugar plus a 40 cc. suspension of 10 g. yeast, fermented at 32.2°C. for 3½ hours—SH reaction negative.

From each of these ferments the yeast was separated in the following manner: The ferments were diluted and strained through a fine bolting silk and the residues washed until the washings were practically free of yeast. The filtrates were then centrifuged until microscopic examination showed that negligible numbers of yeast cells remained in the supernatant liquid. The yeasts thrown down were each mixed with 20 g. of sodium chloride and allowed to stand overnight in order to bring about autolysis of the yeast cells. The autolysates of (*a*) and (*b*) contained, in addition to the autolyzed yeast, some starch and coagulated protein, which would not interfere with the tests. Each of the autolysates was made up to 100 cc. with water and again centrifuged. The liquids so obtained were tested for glutathione SH with sodium nitroprusside. The yeast from the fermented germ suspension (*a*) gave an almost negative reaction, while those from the unfermented germ suspension (*b*) and from the fermented sugar solution (*c*) gave strong positive reactions.

On the assumption that the glutathione originally present in the yeast of the autolysate (*a*) had been oxidized, attempts were made at reduction back to glutathione SH. These attempts failed, showing that the glutathione in the yeast, as well as that in the germ, suffers some more far-reaching change than oxidation to the S–S form.

Summary

It is shown that glutathione is eliminated from raw germ by fermentation.

The effects of germ, raw and fermented, on the properties of dough and bread are shown.

The connection between the presence of glutathione in germ and dough deterioration by germ addition is confirmed.

It is shown that prefermented germ or extracts from prefermented germ have a marked maturing action on doughs.

Evidence is given that an enzyme mechanism is involved in the elimination by fermentation of glutathione from raw germ.

The disappearance of glutathione from fermented germ is not due to oxidation to the S–S form; neither is this the case in aged germ.

The fermentation occurring in fermenting germ suspensions is shown to be an ordinary alcoholic fermentation.

It is shown that when raw germ is fermented, not only the glutathione in the germ but also that in the yeast is eliminated.

The application of the principle of fermenting germ to the making of a germ bread is reported.

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THE DETERMINATION OF FURFURAL-YIELDING SUBSTANCES AND FERMENTABLE CARBOHYDRATES IN GRAIN [1]

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Knowledge of the content of fermentable substances in grain is of prime importance to those industries that ferment grain to alcohol. The research reported here was undertaken in order to establish a means of arriving at the true fermentable content of starch in grain. The acid hydrolysis method for starch was used to determine both fermentable and unfermentable carbohydrates. The rapid bromine method was modified and applied to the determination of the unfermentable carbohydrates, the so-called "pentosans" in grain. The true fermentable substance was obtained by subtracting the pentosan content from the "starch" determination.

[1] Condensed from a thesis presented by Marjorie A. Metzner to the faculty of the Graduate School of the University of Louisville in partial fulfillment of the requirements for the degree of Master of Science, July, 1940.

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In the literature covering the past few decades it has been customary to describe all furfural-yielding substances as pentosans, with the exclusion of the pentoses. The rightfully called pentosans are those substances that on hydrolysis yield the two pentose sugars, arabinose and xylose, and the methylpentose, rhamnose. There are many other naturally occurring substances which, in the course of acid hydrolysis, also yield furfural, especially derivatives of galacturonic and glycuronic acids. The yield of furfural from such a complex mixture as grain, according to Phillips (1940), is derived from various sources which for practical purposes are reckoned as pentosans.

The determination of pentosans consists essentially of conversion by acid to furfural, which is estimated in the distillate by a variety of reagents used in gravimetric, volumetric, and more recently, colorimetric procedures. Almost all of the experimentation in the literature was found to have been directed toward an effort to obtain quantitative yields of furfural from an acid solution. The method most commonly used by agricultural chemists for the determination of pentosans in grain is the phloroglucinol method developed by Tollens (1896). It is a gravimetric procedure and is described in *Official and Tentative Methods of Analysis of the Association of Official Agricultural Chemists.* This method is empirical and time-consuming, and lacks accuracy where very small quantities of furfural are to be determined. The precipitate in this method must be treated with alcohol to extract the alcohol-soluble methylfurfural phloroglucide and hydroxymethylfurfural phloroglucide derived from hexoses from the insoluble furfural phloroglucide in order to obtain the true amount of furfural present. Preece (1940) has questioned this extraction treatment, and expressed a doubt that it achieved any gain in precision. Other precipitants of furfural generally recommended are barbituric acid, thiobarbituric acid, 2,4-dinitrophenylhydrazine and diphenylthiobarbituric acid. In a comparison of the phloroglucinol method, the barbituric method, and the bromine oxidation method, Schmidt-Neilson and Hammer (1932) found that for a pure furfural solution, the bromine method was best.

There are several colorimetric determinations for furfural. Chelintzev and Nikitin (1934) used the orange-red color given with 50% sulfuric acid and furfurylideneacetone formed from acetone and furfural for determinations of furfural in an acid medium in which 1 ml. of a 0.001% solution of furfural gave a definite color. Recently, Reeves and Munro (1940) described an acid hydrolysis of pentoses, followed by extraction with xylene, and determined the furfural colorimetrically with aniline acetate.

The volumetric determinations of furfural are more desirable than the gravimetric because of their advantage of speed and accuracy over

a wide range of concentrations. The use of an excess of bromine to react with furfural and subsequent titration of the unused bromine was proposed by Van Eck (1919) and applied to the determination of pentosans in wood cellulose by Powell and Whittaker (1924). They found an agreement of from 0.20% to 0.01% with the phloroglucinol method. Pervier and Gortner (1923) have described a procedure involving the slow addition of the bromine reagent from a burette until free bromine persisted in the solution as indicated electrometrically. There was difficulty in determining the end-point by this procedure because after one molecule of bromine had reacted with one molecule of furfural there was a tendency for the oxidation product to react further with bromine.

In experiments with furfural from pure xylose, Kline and Acree (1932) studied standard and steam distillation and found that steam distillation gave no better yields of furfural from xylose than the standard method of distillation. They found that the presence of nitric acid or nitrates in the distillation mixture caused destruction of furfural and made necessary the removal of nitrates with nitron. They concluded that the volumetric bromine method is worthy of consideration as an official method. Hughes and Acree (1938, 1939) studied various methods of distillation of solutions of xylose, arabinose, and rhamnose with the object of increasing the yield of furfural and methylfurfural. They employed 12% hydrochloric acid saturated with sodium chloride to accelerate the formation of furfural, which was then removed by steam distillation. They obtained practically theoretical yields of furfural from xylose and arabinose, and methylfurfural from rhamnose.

In the determination of furfural itself with bromine, Hughes and Acree (1934, 1937) have shown that at room temperature the method is less accurate because one molecule of bromine for each molecule of furfural is followed by the slow addition of the second molecule of bromine at 20° to 30°C. Magistad (1933) observed a large temperature coefficient of the second reaction. Hughes and Acree carried out a series of experiments with furfural and bromine at 0°C. in order to determine whether the reaction could be limited to the first step. They found that the reaction of furfural and bromine at 0°C. for 5 minutes gave more accurate results than if left in the dark for one hour at room temperature as in the Powell and Whittaker procedure.

Apparatus and Materials Used

Rye, corn, and barley malts are the principal raw materials for alcohol and whiskey production. Distiller's dried grain is the spent grain recovered after the distillation process. The sample of distiller's dried grain used was processed from the production of stillage (residue of

distillation) from bourbon and grain alcohol in the ratio of 1 to 3, respectively. The bourbon was from a mash bill of 70% corn, 20% rye, and 10% barley malt; the grain alcohol was from a mash bill of 90% corn and 10% distiller's barley malt.

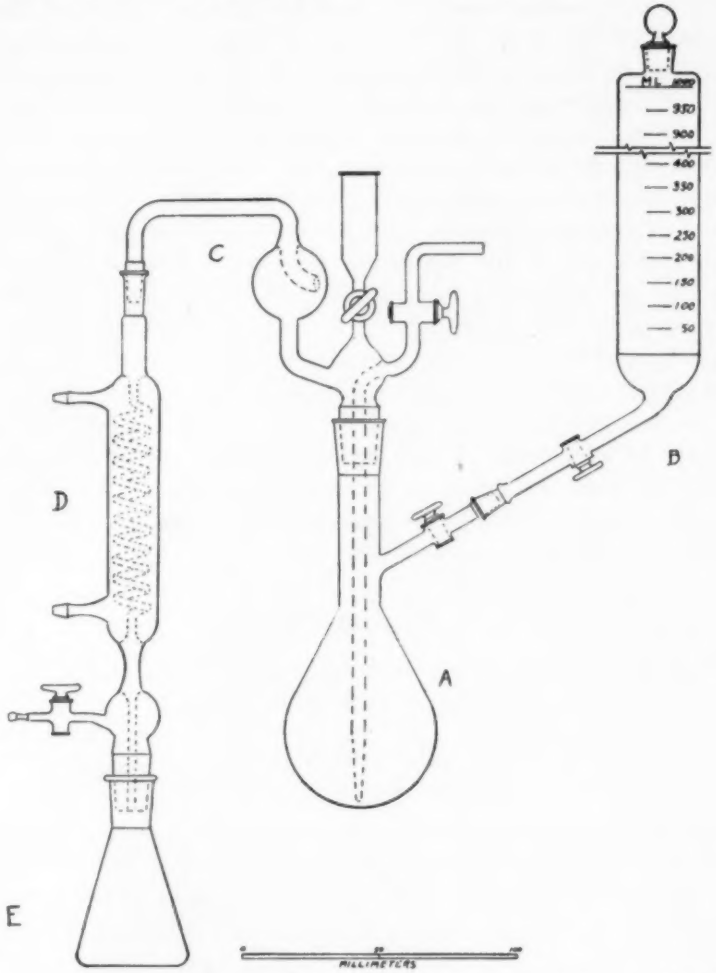

Fig. 1. Apparatus for the determination of pentosans in grain. (Modified apparatus made by the Cincinnati Scientific Company, Cincinnati, Ohio.)

The six samples of corn used were hybrids grown experimentally in the United States corn belt for the purpose of increasing the starch content. Corn and rye starch contents normally run the same but in this case the corn starch was higher than usual. The six samples of rye used were chosen from various sections of the rye belt in the United States and represented the best grade of rye.

The apparatus used was developed by Josef Stastny (1931) for a threefold purpose, namely, for the determination of pentosans in grain, nitrogen traces in spirits, and volatile acids in fermentation products. The apparatus consisted of a Kjeldahl flask (*A*, as shown in Figure 1) with a side arm attachment (*B*) for a burette-type cylinder. Into the Kjeldahl flask was inserted a long tube forming a continuous part of the splash bulb device (*C*) which in turn fitted into a coiled glass condenser (*D*). The condenser was fitted into an Erlenmeyer receiving flask (*E*). For the determination of pentosans in grain a 500-ml. Erlenmeyer receiving flask with ground glass joint was used. This flask was calibrated in divisions of 30 ml. and served as a graduated cylinder for measuring the amount of distillate collected. The burette type of cylinder and double-stopcock delivery to the distillation flask were particularly useful in measuring the amount of hydrochloric acid delivered and in regulating the flow. One of the outstanding features of the apparatus was the ground glass joints. In this particular work the use of ground glass joints was of paramount importance, because when rubber stoppers were used in contact with hot hydrochloric acid vapors, a volatile substance was formed which reacted with bromine. Hughes and Acree (1938) found that when two grams of ground rubber was distilled with 12% hydrochloric acid, 0.32 ml. of 0.1*N* potassium bromate-bromide solution was required by 200 ml. of distillate.

Experimental

Pentosans, during boiling with 12% hydrochloric acid, are hydrolyzed as follows into pentoses and thence to furfural:

$$C_5H_8O_4 + H_2O \rightarrow C_5H_{10}O_5$$
$$C_5H_{10}O_5 \rightarrow C_5H_4O_2 + 3\ H_2O$$

In an acid medium and in the presence of an excess of bromine over a period of one hour at room temperature, one molecule of furfural has been reported to react with four atoms of bromine. The furfural was calculated by the amount of bromine required to saturate it.

$$KBrO_3 + 5\ KBr + 6\ HCl \rightarrow 3\ Br_2 + 6\ KCl + 3\ H_2O$$
$$C_5H_4O_2 + 3\ Br_2 \rightarrow C_5H_4O_2Br_4 + Br_2$$

The excess of bromine was treated with 10% potassium iodide and the liberated iodine immediately titrated with 0.1*N* sodium thiosulfate solution, starch being used as an indicator.

$$2\ KI + Br_2 \rightarrow 2\ KBr + I_2$$
$$2\ Na_2S_2O_3 + I_2 \rightarrow Na_2S_4O_6 + 2\ NaI$$

Known amounts of pure furfural in 12% hydrochloric acid were determined according to the Powell-Whittaker bromate-bromide method. The difference in the percentage of furfural recovered varied from 0.00% to + 2.23%. These results showed the method was inaccurate. The Hughes and Acree bromate-bromide method, calling for the reaction of bromine and furfural at 0°C. for a period of 5 minutes, was next run on samples of pure furfural in hydrochloric acid and the time factor was varied. At reduced temperature and very short intervals of time only one molecule of bromine reacts with a molecule of furfural. The best quantitative results were obtained with a 4-minute period as shown in Table I. The difference in the 1-minute time was so great as

TABLE I

DETERMINATION OF FURFURAL WITH BROMATE-BROMIDE SOLUTION AT 0° C.

Time of reaction	Furfural present	Furfural found	Difference
min.	g.	g.	%
5	0.1241	0.1253	+0.97
5	0.1336	0.1354	+1.4
5	0.1364	0.1382	+1.2
10	0.1417	0.1436	+1.3
10	0.1402	0.1444	+3.0
10	0.1434	0.1463	+2.0
1	0.1382	0.0852	−38.3
1	0.1368	0.0992	−27.5
3	0.1340	0.1235	−7.8
3	0.1460	0.1426	−2.4
3	0.1426	0.1346	−5.6
4	0.1486	0.1486	0.0
4	0.1486	0.1483	−0.2
4	0.1473	0.1472	−0.07

to show the reaction was only approximately three-quarters completed. Evidently at 5 minutes the third atom of bromine had begun to add to furfural and at 3 minutes one molecule of bromine had not yet completely reacted with furfural. At 10 minutes more than one molecule of bromine had reacted. The 4-minute reaction time gave very good checks. Consequently, it was decided to adopt this modification of the Hughes and Acree method and apply it to the determination of pentosans in grain. Pentosan content was run on six samples of corn and six samples of rye and the results as shown in Tables II and III checked within 0.02% to 0.14%.

The method was as follows: An approximately 3-gram sample of finely ground grain was weighed in a tared dish and transferred quantitatively to a 500-ml. flask along with 100 ml. of 12% hydrochloric acid.

TABLE II

DETERMINATION OF PENTOSAN CONTENT OF CORN SAMPLES—MODIFIED BROMATE-BROMIDE METHOD

Sample	Moisture	Pentosan				Difference, dry basis
		Wet basis		Dry basis		
		I	II	I	II	
	%	%	%	%	%	%
1	9.5	5.40	5.43	5.97	6.00	0.03
2	9.5	5.57	5.52	6.15	6.10	0.05
3	9.5	6.03	5.94	6.66	6.56	0.10
4	10.1	5.28	5.37	5.87	5.97	0.10
5	9.5	5.69	5.73	6.29	6.33	0.04
6	10.2	5.76	5.70	6.41	6.35	0.06

TABLE III

DETERMINATION OF PENTOSAN CONTENT OF RYE SAMPLES—MODIFIED BROMATE-BROMIDE METHOD

Origin of sample	Moisture	Pentosan				Difference, dry basis
		Wet basis		Dry basis		
		I	II	I	II	
	%	%	%	%	%	%
Eastern North Dakota	9.36	9.96	9.98	10.99	11.01	0.02
Eastern South Dakota	11.77	9.86	9.97	11.18	11.30	0.12
Central South Dakota	10.69	9.82	9.71	11.00	10.88	0.12
Western North Dakota	10.47	9.01	8.89	10.06	9.93	0.13
Central Minnesota	11.53	9.56	9.43	10.80	10.66	0.14
Northern Minnesota	11.21	9.66	9.56	10.87	10.77	0.10

A few glass beads were added to prevent bumping and the mixture was heated gently at first and eventually so that the distillate was collected at the rate of 30 ml. every ten minutes. From a separatory cylinder 30 ml. of 12% hydrochloric acid was added every 10 minutes to replace the collected distillate. Particular care had to be taken to avoid superheating and decomposition of grain on the walls of the flask. The hydrochloric acid was added, therefore, in such a manner as to wash down the particles adhering to the sides of the flask. After the collection of 270 ml. of distillate in a calibrated flask, the distillation was ended. The total distillate was made up to 300 ml. by the addition of 30 ml. of 12% hydrochloric acid. One hundred ml. of distillate was pipetted into a 150-ml. Erlenmeyer flask, stoppered, and cooled in an ice bath at 0°C. When the bromate-bromide was at this temperature, 25 ml. was pipetted into the distillate and allowed to react with the furfural for 4 minutes. A stop watch or electric time-interval instrument was used so that the

time of reaction would be exact. Occasionally the reaction mixture was shaken. At the end of 4 minutes, 10 ml. of 10% potassium iodide, also at 0°C., was pipetted into the reaction mixture. The flask was shaken well with particular care that no fumes escaped. The contents were titrated with 0.1*N* sodium thiosulfate with starch indicator added at the end of the reaction. A blank was run on 100 ml. of 12% hydrochloric acid in exactly the same manner. The number of milliliters of thiosulfate used for the sample was subtracted from the blank.

Calculation: 1 molecule of thiosulfate was equivalent to 0.0082 g. of pentosan. Number of ml. of 0.1*N* sodium thiosulfate $\times 3 \times 0.0082 =$ grams pentosan present in sample.

$$\frac{\text{Grams pentosan} \times 100}{\text{Weight of sample}} = \%\ \text{pentosans (wet basis)}$$

$$\frac{\%\ \text{pentosans (wet basis)} \times 100}{100.00 - \%\ \text{moisture}} = \%\ \text{pentosans (dry basis)}$$

In order to be satisfactory for general use, the results must check with those obtained by a standard determination. In this case the standard is the phloroglucinol method. Accordingly, pentosan determinations were run on samples of rye, corn, barley malt, and distiller's dried grain by both methods. The results checked very well as shown in Table IV.

TABLE IV

PERCENT PENTOSAN IN GRAIN

Sample	Moisture	Bromate-bromide method					Phloroglucinol method		Difference, dry basis
		Wet basis		Dry basis		Average, dry basis			
		I	II	I	II		Wet basis	Dry basis	
	%	%	%	%	%	%	%	%	%
Rye	8.74	8.78	8.79	9.62	9.63	9.63	8.75	9.59	0.04
Barley malt	8.51	10.53	10.58	11.51	11.57	11.54	10.57	11.55	0.01
Corn [1]	10.10	5.28	5.37	5.87	5.97	5.92	5.39	5.99	0.07
Distiller's dried grain	8.31	13.98	14.17	15.24	15.40	15.32	13.90	15.16	0.16

[1] Same as sample 4 in Table II.

In the volumetric method, distillation was stopped at 270 ml., since a test for furfural in the distillate from the various grains after this amount had been collected proved negative. The test for furfural was conducted as follows: 1 ml. of a mixture of equal portions of aniline, glacial acetic acid, and water gave a characteristic pink test in the distillate prior to the collection of 270 ml. and no color reaction in 10 ml. of the distillate collected after 270 ml. Interfering nitrates were tested

for in the distilling mixture and distillate at intervals of 10 minutes throughout a distillation for each type of grain, and in every case the test showed negative.

The final experiments were carried out to show the true fermentable substances in corn and rye. The starch content of the same six samples of corn and rye was run according to the acid hydrolysis method of the *Official and Tentative Methods of the Association of Official Agricultural Chemists*. The pentosan contents were subtracted from the found starch contents to give a clearer picture of the fermentable substances in the grain. The data are listed in Table V.

TABLE V

STARCH AND PENTOSAN CONTENTS OF RYE AND CORN SAMPLES SHOWING THE TRUE FERMENTABLE SUBSTANCES PRESENT, BY DIFFERENCE

Sample	Starch, dry basis	Pentosan (average), dry basis	True fermentable substances, dry basis
	%	%	%
Rye			
Eastern North Dakota	65.42	11.00	54.42
Eastern South Dakota	65.87	11.24	54.63
Central South Dakota	62.93	10.94	51.99
Western North Dakota	61.02	10.00	51.02
Central Minnesota	64.11	10.73	53.38
Northern Minnesota	63.23	10.82	52.41
Corn			
No. 1	71.12	5.99	65.13
No. 2	70.25	6.13	64.12
No. 3	69.52	6.61	62.91
No. 4	70.70	5.92	64.78
No. 5	71.11	6.31	64.80
No. 6	70.74	6.38	64.36

TABLE VI

CORN

Sample No.	Starch true fermentable substances	Alcohol yield			Difference between columns 4 and 5	Efficiency of fermentation
		Theoretical	Assuming 88% conversion	Actual		
	%	*p.g./bu.*	*p.g./bu.*	*p.g./bu.*	*p.g./bu.*	%
(1)	(2)	(3)	(4)	(5)	(6)	(7)
1	65.13	6.26	5.50	5.17	0.33	94.0
2	64.12	6.16	5.40	5.08	0.32	94.0
3	62.91	6.04	5.30	5.17	0.13	97.5
4	64.78	6.22	5.46	5.17	0.29	94.7
5	64.80	6.22	5.46	5.17	0.29	94.7
6	64.36	6.18	5.42	5.17	0.25	95.4

Each corn sample was fermented with the *Saccharomyces cerevisiae* strain of yeast and the yield of alcohol calculated. Table VI shows calculations of the theoretical proof gallons per bushel obtainable from the established true fermentable substances, the proof gallons per bushel expected with the assumption of 88% starch conversion as practically attainable, the difference in proof gallons per bushel between the yield from 88% starch conversion and the actual yield, and, finally, the efficiency of the fermentation. The starch content determined polarimetrically on these same samples in every case was higher than that of the true fermentable starch content, and fermentation efficiency calculated on this starch basis would be much lower than that shown in Table VI.

Discussion

By determining the pentosan content of grain and subtracting this figure from the starch content obtained by acid hydrolysis, a somewhat truer picture of the fermentable substances present in the grain was drawn than was otherwise possible by present methods. It must be held in mind that the term " pentosan " is used to include also other furfural-yielding materials. The hydrolysis products of these substances in addition to the converted starch reduced Fehling's solution and the total was expressed as dextrose, which was calculated back to starch. The pentosan content was then subtracted from this starch figure and the remainder was the fermentable figure.

The fermentable figure provides the chemist with a tool in assessing the value of grain. The utilization of this tool has been facilitated by the application of a rapid, modified bromine method for the determination of pentosans in grain and of an apparatus set-up especially designed for this purpose. The speed and accuracy of the method lend it to practical, industrial usage.

The modified excess-bromine method checked very well with the standard and itself as shown by duplicate runs on every sample. It is, therefore, worthy of consideration as an official method. It is more rapid than the standard method by approximately 38 hours and is accurate over a wider range of concentrations. A complete determination took about 100 minutes, which was even more rapid than that reported recently by Reeves and Munro (1940). The refluxing and extraction involved in their pentose determination consumed 150 minutes. A number of workers have reported that amounts of furfural less than 0.01 g. were not quantitatively determined by the precipitation method which either failed to indicate the presence of any furfural below this concentration or only a small fraction thereof, whereas the excess-bromine titration method was sensitive to such a small amount.

Sources of error in pentosan determinations such as loss of furfural by volatilization, decomposition of furfural by local superheating, and the use of rubber stoppers were avoided by the use of the special apparatus. The standard method of distillation allowed the distillate to drop from the end of the condenser into the receiver without special precaution against loss by volatilization. In the special apparatus the distillate was closed to air until the actual titration.

Corn and rye usually give approximately the same starch analysis by acid hydrolysis but the alcohol recovered by yeast fermentation from rye was much lower in comparison than that from corn. This fact was due to the presence of more substances unfermentable by yeast enzymes in rye than in corn as the analyses show in Table V. Control starch and pentosan analyses on grain are very important as shown by variations as much as 4.85% and 1.25%, respectively, on rye from eastern South Dakota and western North Dakota.

Rye from eastern North Dakota and eastern South Dakota was shown to contain the greatest amount of true fermentable substances.

The study of alcoholic fermentation made by Pasteur resulted in an alcoholic fermentation balance that was so authoritative that to date it has not been changed, although much controversy has arisen over it as presented in papers by Savary (1938, 1939) and Perard (1939). Pasteur found an alcoholic fermentation of cane sugar to yield alcohol, carbonic acid, glycerin, and succinic acid. It is not possible to convert starch completely to alcohol and an efficiency fermentation of 95%, based on the theoretical and actual yields, is optimum, according to this fermentation balance.

When we consider that all this work was empirical and that the study of truly quantitative analyses of the composition of grain is far from perfected, an effort toward the finding of satisfactory analytical methods would be well worth while. The yield of furfural, for example, was from a complex mixture and was derived from many sources. It was impossible to correlate the percentage of furfural obtained thus with the percentage of pure pentosan in the grain. In the starch acid hydrolysis method, the hydrolysate may have consisted of a mixture of hexoses, pentoses, uronic acids, and possibly other substances capable of reducing Fehling's solution. The reducing value obtained was the resultant of interacting and opposing effects and was expressed as dextrose. This value was translated to an actual term of starch, and by subtracting the value obtained for "pentosan" content we arrived at the empirical true content of starch. Preece (1931), in attempting to determine hemicelluloses, actually isolated and weighed them by a tedious and rather inaccurate method. Anderson and Krznarich (1935) and Krznarich (1940) have separated the hemicelluloses from oat hulls into

further components by repeated fractionation. These direct determinations are deserving of further study.

With our empirical methods, we do nonetheless obtain comparable data that are of great value.

Summary

The reaction of furfural in the distillate from 12% hydrochloric-acid distillation of grain with bromine at 0°C. over a period of 4 minutes was limited to the addition of one molecule of furfural to one molecule of bromine. Of several time intervals for the reaction to proceed, the 4-minute period proved optimum. The contents of original unfermentable substances in various grain samples were determined from the amount of furfural in the distillate, which was calculated from the determination of excess bromine in solution titrimetrically with sodium thiosulfate. This method checked very well with the standard phloroglucinol one for the determination of pentosans in grain. The application and advantages of a special apparatus for the determination of pentosans in grain are shown. A true representation of fermentable substances in grain has been established by subtracting this unfermentable content from the starch content obtained by the acid-hydrolysis method, which included both fermentable and unfermentable carbohydrates. This fermentable figure serves as an important tool for practical industrial control.

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THE COMPARATIVE BAKING QUALITIES OF STARCHES PREPARED FROM DIFFERENT WHEAT VARIETIES

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Alsberg (1935) mentioned the influence that starch might have upon flour quality and the need for investigational work directed toward a solution of this problem. As pointed out in a publication by Harris and Mason (1940) the starch in wheat flour has not received the consideration that it deserves from the standpoint of flour quality. This constituent is normally present in wheat flour in a concentration of at least 70% by weight and it is only reasonable to expect that some effect must be exerted upon baking strength by variations in starch properties due to wheat variety or environmental conditions during the growing season of the plant when the starch is being elaborated. To correlate starch differences with baking quality has been rather difficult, owing to the effect of other variables that are operative, such as wheat gluten, which is a very important factor in relation to baking strength. A number of papers have been published dealing with the results of viscosity, phosphorus, swelling, diastatic, and other determinations upon starches prepared from different wheats. Efforts have been made to correlate the results with flour quality. This work has been reviewed by Harris and Mason (1940) and will not be cited in the present instance. These studies, however, have been handicapped by the presence of other variables in the baking test that influence the quality of the loaf produced.

Sandstedt, Jolitz, and Blish (1939) published the results of a study conducted to ascertain the possibility of producing bread from synthetic doughs made from mixtures of gluten and starch with the usual baking ingredients. These researchers prepared gluten and starch from wheat flour and mixed them in suitable proportions to produce satisfactory bread. The doughs were mixed in the Hobart-Swanson with water, yeast, sugar, and salt in the usual proportions with the exception of sugar, which was present in 7% concentration. The doughs were fermented and baked, producing loaves of the customary size and appearance. The additional quantity of sugar was included in the formula to prevent yeast starvation during the fermentation period. This method, it would seem, could be employed, as pointed out by the authors, for the direct determination of starch quality effects upon baking strength since a uniform gluten substrate could

be used upon which different starches might be superimposed in a series of doughs. Differences in baking results obtained could then be attributed to variations in baking quality of the starches employed in making up the doughs.

Experimental Material and Methods

Starches were prepared from 20 flours which had been experimentally milled from varieties of the hard red spring, hard red winter, soft red winter, durum, and white wheat classes. These wheats were grown in the states of North Dakota, Kansas, Nebraska, Indiana, and Washington. The starch was separated from the gluten by washing under a small stream of 0.1% sodium phosphate solution. The washings containing the starch were strained through a 48-XX bolting cloth followed by a second straining through a 12-XX silk. In the latter instance, some time was required to work the starch suspension through the cloth. About 2,000 cc. of suspension was washed on the average from 400 g. of flour. The quantity of flour used varied from 248 g. to 400 g., depending upon the amount available for the investigation.

After the double straining, the starch was allowed to settle out in the cold and the supernatant liquid decanted from the residual starch. The starch was centrifuged, and the separated liquid again decanted from the moist starch and a supernatant gelatinous layer. This layer was tentatively named "amylodextrin" by Sandstedt, Jolitz, and Blish (1939)—inasmuch as it appeared that this material had been formed from soluble starch by the action of beta-amylase. The same term will be used to denote this substance in the present paper. The starch and amylodextrin were removed from the centrifuge tubes and dried in trays under a fan at 90° to 94°F. with occasional turning and pressing with a spatula, to facilitate the removal of moisture and to break up the lumps which formed during drying. The dried starch was finally run through a small wheat grinder to reduce any lumps which had formed while the starch was drying. The sample was thoroughly mixed and the moisture and nitrogen content determined.

A series of preliminary experiments was made to determine the best method to use for the purpose of obtaining optimum baking results. It was decided to omit entirely the sponge procedure of preparation and to investigate the "soaker" method. Both of these methods were developed by Sandstedt, Jolitz, and Blish (1939). The procedures used and the baking results yielded by these variations of gluten treatment and mixing times are shown in Table I. The first attempt made to incorporate dry gluten and starch into a dough without an initial period of gluten hydration resulted in an inferior

TABLE I

METHOD OF GLUTEN HYDRATION AND MIXING TIMES EMPLOYED IN THE PRELIMINARY STUDY INCLUDING BAKING DATA

Protein level of starch-gluten mixture 13.2% (13.5% moisture basis).

No. of procedure	Method employed: Gluten hydrated at 30°C.	Mixing time	Loaf volume	Texture[1]	Crumb color[2]	Crust[3]	Symmetry[4]
		min.	*cc.*				
I	Gluten and starch mixed dry.	2½	150	5.0 c	5.0 g-y	S	2.5
II	Gluten worked into a ball with a quantity of water and allowed to soak 1 hour; then excess water squeezed out, ball broken into small pieces and mixed with the starch.	1	155	5.5 C, o	5.0 g-y	Du	3.5
		2	165	6.5 o	6.0 g-y	S	4.0
		2½	200	7.0	7.0	S	4.5
III	12 cc. of water added to the gluten in a 30-cc. beaker and allowed to soak (without agitation) for 1 hour; formed into ball; pressed out flat and cut into long thin strips and mixed with the starch.	1	165	6.0 C, o	5.0 g-y	Du	3.5
		2	185	6.5 o	6.0 g-y	S	4.0
		2½	205	7.5	7.0	S	4.5

[1] Texture: o = open, C = coarse, c = close. Perfect score = 10.
[2] Color: g-y = gray-yellow. Perfect score = 10.
[3] Crust: Du = dull, S = satisfactory.
[4] Symmetry: Perfect score = 5.

loaf. The next method tried involved forming a gluten ball which stood for one hour at 30°C. The ball was then broken into small pieces and mixed with the starch. Mixing periods of 1 to 2½ minutes were used, the best results being obtained with the 2½-minute mix. The next trial was made by adding a definite quantity (12 cc.) of distilled water to the gluten and soaking for one hour. The hydrated gluten was then formed into a ball with the fingers, pressed flat and cut into strips which were incorporated with the dry starch into a dough. In this instance, 2½ minutes of mixing again gave optimum results. As this method of gluten hydration with 2½-minute mixing gave the best results, it was adopted for use in the starch-quality investigations. The use of periods shorter than one hour for gluten hydration resulted in a "granular" feeling dough, in which the original gluten particles could be observed, indicating apparently imperfect gluten hydration.

The Hobart mixer, equipped with two dough hooks, was used for mixing the synthetic doughs, employing the medium speed for 30 seconds. The mixer was then stopped and the gluten and starch scraped down from the sides of the bowl and the mixing pins. Fifteen seconds was allowed for this operation. The mixer was again started and low-speed mixing conducted until the stop watch registered a total of 2½ minutes from the time when the mixer was first started. Thus

a total mixing time of 2½ minutes, less 15 seconds, was used. The gluten and starch were found to mix quite readily and no indications were noticed of incomplete incorporation of the gluten in the dough. The loaves were quite normal in appearance, color, and texture. The micro baking method described by Geddes and Aitken (1935) and used by Harris and Sanderson (1939), as well as Van Scoyk (1937, 1939), was employed in this investigation, and proved to be quite satisfactory from the standpoint of results obtained. This method was especially suitable because of the relatively small quantities of starch and gluten required to produce a dough as compared with the older methods which necessitate the use of much larger doughs. The formula used was the malt-phosphate-bromate with 7% of sucrose included to insure the presence of sufficient fermentable material during the dough-fermentation period. A standard period of 3 hours of fermentation previous to panning with 55 minutes in the pan was allowed. The usual methods of handling the dough were followed.

The dried, powdered gluten used in this study was prepared from a commercial hard red spring wheat flour (13.2% protein) by the method described by Harris (1940). This gluten contained 10.1% moisture and 69.8% crude protein (N × 5.7) on a 13.5% moisture basis. Gluten in this form is more convenient to use than wet crude gluten, as pointed out by Aitken and Geddes (1938) and also by Harris (1940), although some evidence of alteration in the biochemical properties of the gluten occasioned by drying have been found by the latter.

Three protein levels, 10.0%, 13.2%, and 16.0% (13.5% moisture basis), were selected for the starch-gluten mixtures. It is probable that the gluten protein content of these levels would be somewhat above that of flours of equivalent total protein content because of the inclusion of other than gluten protein in the latter value. The proportions of prepared dried starch and gluten necessary to produce a synthetic flour at these three protein contents were calculated, taking into account the respective protein contents of the dried gluten and starch. The quantity of gluten required for each dough was weighed out and placed in a small beaker under distilled water for one hour. For the lower protein levels of 10.0% and 13.2%, 12 cc. of distilled water was used. In the instance of the 16.0% protein level, 15 cc. of distilled water was used. All water added was at a temperature of 30°C. and allowed to stand for one hour to hydrate the gluten. The necessary quantity of starch was weighed out and placed in a suitably numbered container. The container and contents were then placed in a cabinet at 30°C. until required for mixing with the gluten. The entire quantity of water added to the glutens was later transferred to the mixing bowl.

Discussion of Results

A description of the wheats from which the starches were prepared with comparative milling and analytical data are presented in Table II. The wheat protein covered a range of 7.6%, while the flour

TABLE II

DESCRIPTIONS AND COMPARATIVE DATA OBTAINED ON THE WHEATS FROM WHICH STARCHES USED IN THE BAKING INVESTIGATIONS WERE PREPARED

Results arranged in order of increasing wheat protein content. All samples of wheat grown in 1939 except Nos. 1, 5, 7, and 20, which were grown in 1938.

Sample No.	Class and variety[1]	State grown	Test weight per bushel	Crude protein (N ×5.7)		Total flour yield	Flour ash
				Wheat	Flour		
			lbs.	%	%	%	%
1	SRW, Trumbull	Ind.	63.8	10.0	8.5	72.3	0.48
2	SRW, Wabash	Ind.	64.2	10.1	8.6	66.6	0.48
3	SW, Federation	Wash.	60.8	10.8	9.3	66.0	0.40
4	HW, Early Baart	Wash.	63.5	11.1	9.7	65.9	0.44
5	HRW, Chiefkan	Kan.	62.7	11.2	10.5	76.3	0.48
6	HRS, Vesta	N. D.	61.2	11.6	11.3	73.5	0.48
7	HRW, Kanred	Kan.	58.0	12.8	12.3	74.0	0.51
8	HRW, Tenmarq	Kan.	61.5	14.1	13.2	63.3	0.38
9	HRS, Mercury	N. D.	56.3	14.2	13.4	70.2	0.56
10	HRS, Rival	N. D.	55.5	15.2	14.0	70.6	0.43
11	E, Vernal	N. D.	31.8	15.2	16.8	47.2	0.68
12	D, Mindum	N. D.	60.2	15.3	14.6	69.9	0.56
13	HRS, Premier	N. D.	58.2	15.3	15.0	70.3	0.54
14	HRW, Chiefkan	Kan.	61.8	15.8	15.3	73.0	0.43
15	HRW, Cheyenne	Nebr.	63.2	16.2	14.6	63.5	0.47
16	HRW, Blackhull	Kan.	61.0	16.2	15.8	68.5	0.45
17	HRW, Nebred	Nebr.	59.8	16.9	16.2	68.3	0.42
18	HRS, Thatcher	N. D.	56.6	16.9	16.6	69.0	0.51
19	HRW, Turkey	Kan.	61.5	17.2	16.9	69.5	0.58
20	D, Mindum	N. D.	60.8	17.6	17.1	75.6	0.88

[1] SRW = soft red winter. SW = soft white. HRW = hard red winter. HRS = hard red spring. E = emmer. D = durum.

protein covered a range of 8.6%. There was also a marked variability in total flour yield, as would be expected with milling wheats as variable in nature as those encountered in this investigation. It will be noticed that the Tenmarq from Kansas produced the lowest-ash flour, while the new Nebred, grown in Nebraska, produced the second-lowest. Several of the wheats produced relatively high-ash flours, such as Vernal emmer, the two samples of durum grown in North Dakota, and Turkey from Kansas.

In Table III are shown the comparative baking data obtained on the flours. The absorption shows substantial differences between the various samples, being lowest for Vernal emmer and highest for Mindum. The soft winter wheats, as a whole, ran lower than the hard wheats in water-absorbing capacity. The loaf volumes varied from

TABLE III

COMPARATIVE BAKING DATA OBTAINED ON THE FLOURS FROM WHICH THE STARCHES WERE PREPARED

Results arranged in order of increasing wheat protein content.

Sample No.	Class and variety[1]	Absorption	Loaf volume	Texture[2]	Crumb color[3]	Crust[4]	Symmetry[5]
		%	cc.				
1	SRW, Trumbull	58.0	469	7.4	7.2	P	2.5 o
2	SRW, Wabash	51.3	485	6.5 o	7.0 y	P	2.5 o
3	SW, Federation	52.2	440	3.5 C, o	5.0 g-y	P	2.0 o
4	HW, Early Baart	55.6	510	6.5 o	7.0 y	P	2.5 o
5	HRW, Chiefkan	58.0	510	3.0 C, o	5.0 g-y	Du	2.5 o
6	HRS, Vesta	57.8	490	6.0 C, o	6.8 y	S	3.0 o
7	HRW, Kanred	58.0	616	7.2	7.3	S	3.5 o
8	HRW, Tenmarq	54.0	595	7.5	8.2	S	4.0 o
9	HRS, Mercury	58.1	680	7.1	7.3	SlD	4.4 o
10	HRS, Rival	59.6	685	7.5	7.8	D	4.3 o
11	E, Vernal	49.8	510	3.0 C, o	3.5 g	Du	2.0
12	D, Mindum	54.6	490	4.5 C, o	4.5 y	Du	2.5 o
13	HRS, Premier	58.1	685	7.5	7.5	SlD	4.5 o
14	HRW, Chiefkan	58.4	655	6.5 o	7.5	S	4.2 o
15	HRW, Cheyenne	55.1	655	7.2	7.8	S	4.0 o
16	HRW, Blackhull	55.7	715	7.0 o	7.8	S	4.5 o
17	HRW, Nebred	53.8	780	7.0 o	7.2	S	4.5 o
18	HRS, Thatcher	59.6	910	6.2 o	7.0 y	SlD	4.5 o
19	HRW, Turkey	55.3	720	6.5 o	7.0 y	S	4.5 o
20	D, Mindum	63.0	465	7.3	7.0	Du	2.5 o

[1] See footnote, Table II.
[2] Texture: o = open, C = coarse, c = close. Perfect score = 10.
[3] Color: y = yellow, g-y = gray-yellow, g = gray. Perfect score = 10.
[4] Crust: Du = dull, S = satisfactory, SlD = slightly dark, P = pale.
[5] Symmetry: o = overoxidized. Perfect score = 5.

440 to 910 cc., a range of 470 cc., while other characteristics of the loaves likewise showed large variations between the various flours. A series of flours milled from wheats embracing such disparity in baking strength and grown under marked differences of environmental conditions, should form excellent material for the preparation of starches to be used in a study of the effects of starch differences upon baking strength. If wheat starches differ in baking characteristics, these differences should be apparent in a study conducted with variable material of this nature.

It may be pointed out that several of the hard red spring wheats included in this investigation are quite new. Mercury is one of these and has been found to be below the average of spring wheat in baking quality and has, accordingly, not been distributed to the grain growers of North Dakota. Vesta is another new variety that has been under consideration for distribution, but has not yet been recommended. Premier is a third recently developed variety, and no decision has been made in respect to its release. Rival is considered to be higher in baking strength than the other spring wheats listed, with the exception

of Thatcher, which is a very strong wheat. From results obtained in this laboratory when the 1939 crop was examined, these wheats would rank as follows in order of decreasing baking strength: Thatcher, Rival, Vesta, Premier, and Mercury.

In Table IV are shown the moisture and protein contents of the starches prepared from this series of wheats. A substantial variation

TABLE IV

CLASS AND VARIETY OF WHEAT FROM WHICH STARCH WAS PREPARED AND MOISTURE AND PROTEIN CONTENT OF STARCH

Sample No.	Class and variety	Starch	
		Moisture	Protein[1]
		%	%
1	Soft red winter, Trumbull	10.8	0.44
2	Soft red winter, Wabash	10.8	0.49
3	Soft white, Federation	10.9	0.34
4	Hard white, Early Baart	11.1	0.40
5	Hard red winter, Chiefkan	11.9	0.48
6	Hard red spring, Vesta	12.1	0.47
7	Hard red winter, Kanred	10.8	0.52
8	Hard red winter, Tenmarq	10.6	0.45
9	Hard red spring, Mercury	11.5	0.71
10	Hard red spring, Rival	11.8	0.59
11	Emmer, Vernal	9.0	0.45
12	Durum, Mindum	9.0	0.56
13	Hard red spring, Premier	12.5	0.53
14	Hard red winter, Chiefkan	8.9	0.44
15	Hard red winter, Cheyenne	10.6	0.52
16	Hard red winter, Blackhull	9.6	0.41
17	Hard red winter, Nebred	9.1	0.51
18	Hard red spring, Thatcher	13.6	0.65
19	Hard red winter, Turkey	8.9	0.51
20	Durum, Mindum	10.9	0.83

[1] Protein calculated (N × 5.7) on 13.5% moisture basis.

in moisture content is noticeable among the various starch samples, ranging from 9.0% to 12.5%—a difference of 3.5%, but whether this is due to the inherent characteristics of the starch is somewhat doubtful as no particular care was taken during drying to insure a constant moisture level in the samples. There does seem to be some indication, however, of a higher moisture content in the starches prepared from the hard red spring wheats. The protein content of the starches was as constant as one would expect, considering the method of preparation. No attempt was made to extract any residual protein from the starches by the use of protein solvents.

Table V presents the comparative baking data obtained from the synthetic starch-gluten doughs mixed and baked as described. These data are on a 13.5% moisture basis and the samples have been arranged in order of decreasing loaf volume within classes to correspond with the

photographs of the loaves. The average loaf volumes and loaf scores are presented in Table VI. The absorption tended to increase with the protein level of the dough as would be expected. The 16.0%-protein-level doughs were very high in absorption, ranging up to 77.7% for the blend made with Mindum wheat starch, sample No. 20. The two durum wheat starches produced doughs with the highest absorptions. It is also quite clear that loaf colume increased with protein content in every instance, showing that in synthetic doughs made from dried starch and gluten, protein content is an important

TABLE V

BAKING DATA OBTAINED FROM SYNTHETIC STARCH-GLUTEN DOUGHS AT THREE PROTEIN LEVELS

Data arranged in order of decreasing loaf volume within classes.

Sample No.	Variety	Absorption—protein levels of			Loaf volumes—protein levels of			Texture[1]—protein levels of		
		10.0%	13.2%	16.0%	10.0%	13.2%	16.0%	10.0%	13.2%	16.0%
					cc.	cc.	cc.			
	HARD RED SPRING VARIETIES									
18	Thatcher	60.7	61.7	63.7	165	185	215	5.0 C, o	5.0 C, o	5.5 o
10	Rival	64.7	64.7	67.7	145	165	210	7.0	7.0	7.0
9	Mercury	63.7	63.7	66.7	145	170	205	4.5 c	6.0	7.0
13	Premier	63.7	63.7	66.7	150	165	195	6.0	6.0	7.0
6	Vesta	63.7	63.7	66.7	105	155	170	3.5 c	4.5 c	6.0
	HARD RED WINTER VARIETIES									
17	Nebred	65.7	67.7	69.7	142	175	205	5.5 o	6.5 o	7.0
15	Cheyenne	67.7	69.7	71.7	140	155	200	5.5 o	6.5 o	7.0
16	Blackhull	66.7	67.7	69.7	135	165	180	4.5 c	6.5 o	6.5 o
19	Turkey	67.7	69.7	71.7	125	155	180	4.5 c	5.5 c	6.0
14	Chiefkan	67.7	69.7	71.7	135	155	175	4.5 o	6.5 o	7.0
8	Tenmarq	63.7	69.7	71.7	120	155	165	4.5 c	5.5 c	6.5 o
	MISCELLANEOUS VARIETIES									
2	Wabash	62.7	64.7	68.7	178	210	255	6.5 o	7.0	7.0
11	Vernal emmer	69.7	69.7	71.7	150	172	215	5.5 o	6.5 o	7.0
12	Mindum	68.7	72.7	73.7	132	165	200	4.5 c	6.5 o	7.0
3	Federation	61.7	63.7	67.7	135	155	185	4.5 c	6.5 o	7.0
4	Early Baart	63.7	63.7	67.7	125	152	175	4.5 c	5.5 c	6.0 o
	VARIETIES GROWN IN 1938									
7	Kanred	66.7	67.7	69.7	158	185	215	5.5 o	6.5 o	7.0
5	Chiefkan	71.7	71.7	71.7	150	175	215	5.5 o	7.0 o	6.5 o
1	Trumbull	65.7	67.7	69.7	140	195	200	5.5 o	7.0	7.0
20	Mindum	75.7	77.7	77.7	130	150	198	4.5 c	6.5 o	7.0

TABLE V—*Continued*

Sample No.	Variety	Crumb color[2]—protein levels of			Crust color[3]—protein levels of			Symmetry[4]—protein levels of		
		10.0%	13.2%	16.0%	10.0%	13.2%	16.0%	10.0%	13.2%	16.0%
	HARD RED SPRING VARIETIES									
18	Thatcher	4.5 g-y	5.5 g-y	6.0 g-y	Du	S	S	2.0 o	3.0 o	4.5 o
10	Rival	6.0 g-y	6.5 g-y	6.5 g-y	P	S	S	2.0 o	4.0 o	4.5 o
9	Mercury	5.5 g-y	5.5 g-y	6.5 g-y	P	Du	S	2.0 o	3.0 o	4.5 o
13	Premier	5.0 g-y	6.0 g-y	6.5 g-y	P	Du	S	2.0 o	3.0 o	4.5 o
6	Vesta	4.0 g-y	5.5 g-y	5.5 g-y	P	Du	Du	1.0 o	2.0 o	4.0 o
	HARD RED WINTER VARIETIES									
17	Nebred	6.0 g-y	6.5 g-y	6.5 g-y	P	Du	S	2.0 o	3.5 o	4.5 o
15	Cheyenne	5.5 g-y	6.5 g-y	6.5 g-y	P	S	S	2.0 o	3.0 o	4.5 o
16	Blackhull	5.5 g-y	6.5 g-y	6.5 g-y	P	S	S	1.0 o	3.5 o	3.5 o
19	Turkey	6.0 g-y	6.0 g-y	6.5 g-y	P	P & Du	Du	1.0 o	2.5 o	3.5 o
14	Chiefkan	6.0 g-y	6.5 g-y	6.5 g-y	P	Du	S	2.0 o	2.5 o	3.5 o
8	Tenmarq	5.0 g-y	6.0 g-y	6.0 g-y	P	P & Du	Du	1.0 o	2.5 o	3.5 o
	MISCELLANEOUS VARIETIES									
2	Wabash	6.5 g-y	6.5 g-y	7.0 y	Du	S	S	4.0 o	4.0 o	4.5 o
11	Vernal emmer	6.0 g-y	6.5 g-y	6.5 g-y	S	Du	S	3.5 o	3.5 o	4.5 o
12	Mindum	6.0 g-y	6.5 g-y	6.5 g-y	Du	Du	S	2.0 o	3.5 o	4.0 o
3	Federation	6.5 g-y	6.5 g-y	6.0 g-y	P	P & Du	Du	2.0 o	3.0 o	4.0 o
4	Early Baart	5.0 g-y	6.0 g-y	6.0 g-y	P	P & Du	Du	1.0 o	3.0 o	4.0 o
	VARIETIES GROWN IN 1938									
7	Kanred	6.5 g-y	7.0 y	7.0 y	Du	S	S	3.5 o	3.5 o	4.5 o
5	Chiefkan	5.5 g-y	7.0 y	6.5 g-y	S	S	S	3.0 o	4.0 o	4.5 o
1	Trumbull	6.5 g-y	6.5 g-y	6.5 g-y	P	Du	S	2.0 o	4.0 o	4.5 o
20	Mindum	5.5 g-y	6.5 g-y	6.5 g-y	Du	S	S	2.0 o	3.0 o	3.5 o

[1] Texture: o = open, C = coarse, c = close. Perfect score = 10.
[2] Color: y = yellow, g-y = gray-yellow, g = gray. Perfect score = 10.
[3] Crust: Du = dull, S = satisfactory, SlD = slightly dark, P = pale.
[4] Symmetry: o = overoxidized. Perfect score = 5.

factor in determining the loaf volume. The protein added was, of course, the same sample throughout and therefore differences due to variations in protein *quality* are eliminated. The texture and symmetry scores were also increased by increasing the gluten content.

The highest loaf volume at all protein levels was produced by starch from a soft red winter wheat, Wabash. These loaves were of excellent crumb color, texture, and symmetry. The doughs mixed from the starch from this variety were firm and elastic and showed every indication during handling of producing superior bread. The starch next in order from the standpoint of baking performance was prepared from wheat No. 18, Thatcher, a hard red spring variety,

TABLE VI

AVERAGE ABSORPTION AND LOAF VOLUME WITH CRUMB COLOR, TEXTURE, AND SYMMETRY SCORES OF STARCH-GLUTEN BLENDS

Data arranged in order of decreasing loaf volume within classes.

Sample No.	Variety	Absorption	Loaf volume	Texture[1]	Crumb color[1]	Crust color[1]	Symmetry[1]
		%	cc.				
	HARD RED SPRING VARIETIES						
18	Thatcher	62.0	188	5.2 C, o	5.3 g-y	S	3.2 o
10	Rival	65.7	173	7.0	6.3 g-y	S	3.5 o
9	Mercury	64.7	173	5.8 o	5.8 g-y	Du	3.2 o
13	Premier	64.7	170	6.3	5.8 g-y	Du	3.2 o
6	Vesta	65.0	143	4.7 c	5.0 g-y	Du	2.3 o
	HARD RED WINTER VARIETIES						
17	Nebred	67.7	174	6.3 o	6.3 g-y	Du	3.3 o
15	Cheyenne	69.7	165	6.3 o	6.2 g-y	S	3.2 o
16	Blackhull	68.0	160	5.8 o	6.2 g-y	S	2.7 o
19	Turkey	69.7	153	5.3 c	6.2 g-y	Du	2.3 o
14	Chiefkan	69.7	155	6.0 o	6.3 g-y	Du	2.7 o
8	Tenmarq	68.4	147	5.5 c	5.7 g-y	Du	2.3 o
	MISCELLANEOUS VARIETIES						
2	Wabash	65.4	214	6.8 o	6.7 g-y	S	4.3 o
11	Vernal emmer	70.4	179	6.3 o	6.3 g-y	Du	3.8 o
12	Mindum	71.7	166	6.0 o	6.3 g-y	Du	3.2 o
3	Federation	64.4	158	6.0 o	6.3 g-y	Du	3.0 o
4	Early Baart	65.0	151	5.3 c	5.7 g-y	Du	2.7 o
	WHEATS GROWN IN 1938						
7	Kanred	68.0	186	6.3 o	6.8 y	S	3.8 o
5	Chiefkan	71.7	180	6.3 o	6.3 g-y	S	3.8 o
1	Trumbull	67.7	178	6.5 o	6.5 g-y	Du	3.5 o
20	Mindum	77.0	159	6.0 o	6.2 g-y	S	2.8 o

[1] For explanation of letters used in baking scores, refer to footnote on Table V.

with Kanred starch next in quality ranking. Vernal emmer starch produced an excellent loaf at the higher protein concentrations. The dough made from the Thatcher starch blends comported itself quite differently from the doughs prepared from other starch samples in the baking test, forming a sticky, unsatisfactory dough which gradually became firm and more consistent as the fermentation progressed. In this manner the dough was similar to doughs made from certain samples of spring wheat flour that are slack and loose after mixing but which tighten up and produce satisfactory loaves of bread. Another feature of this investigation was the excellent loaves produced by starch

prepared from the hard red winter variety, Chiefkan, which was grown in 1938. The other Chiefkan sample, No. 14, grown in 1939, produced a starch distinctly lower in baking quality as judged by these tests. Vesta wheat starch was comparatively poor in performance, with Tenmarq, Early Baart, and Turkey increasing in value in the order named. Mindum wheat produced in 1938 and 1939 did not show great differences in starch quality in these tests.

Photographs of the loaves produced from the different starch-gluten blends are shown in Figures 1 to 8. These photographs, which are arranged in order of decreasing loaf volume by classes, emphasize the points already brought out in this discussion.

Figures 1 and 2 show the exterior and interior appearance of the loaves baked from starches prepared from hard red spring wheat varieties and dried gluten. In the higher protein range the loaves were of a strong, bold appearance with an even break and shred. In the instance of the lower protein levels the loaves had an overoxidized appearance, but this characteristic disappeared when the protein content was increased to 16.0%. Thatcher wheat starch produced loaves with a tendency toward an open texture, but the loaf was quite elastic with thin cell walls; the other hard red spring wheat starches yielded loaves that were better than Thatcher in texture. Vesta starch baked into the poorest loaf shown in Figures 1 and 2, especially at the 10.0% protein level. The crumb color was very good for all the loaves with the exception of Vesta, which was inferior.

The loaves baked from the hard red winter starch blends are shown in Figures 3 and 4. These starch-gluten blends all produced bold, symmetrical loaves at the 16.0% protein level. Loaves made from Nebred and Cheyenne were the strongest in appearance. The crust was very satisfactory except in the instance of Turkey and Tenmarq, which had a dull sheen. The textures were all good in the high protein blends, and the crumb colors could be classified as fair.

The loaves obtained from blends made with starches from a miscellaneous group of varieties are shown in Figures 5 and 6. As pointed out in the instance of the spring-wheat varieties, the loaves from the high-protein blends lack the overoxidized appearance of the lower-protein blends. The loaves in the high-protein level were bold, with an even break and shred. The crust color was satisfactory except for the loaves prepared from the white-wheat starches, which were decidedly dull. Textures were good in this group. The crumb colors were on a lower level than in the preceding groups. Remarkably large, bold loaves were produced from the Wabash-starch blends.

Figures 7 and 8 show loaves baked from hard and soft red winter and white-wheat starch blends. It is interesting to note that the two

blends of soft-wheat starch produced larger loaves than the two blends of hard-wheat starch at all protein levels. In the case of the red winters the soft-wheat blends exceeded the hard-wheat-starch doughs in loaf volume by 58 cc. at the 10% level, by 55 cc. at the 13.2% level,

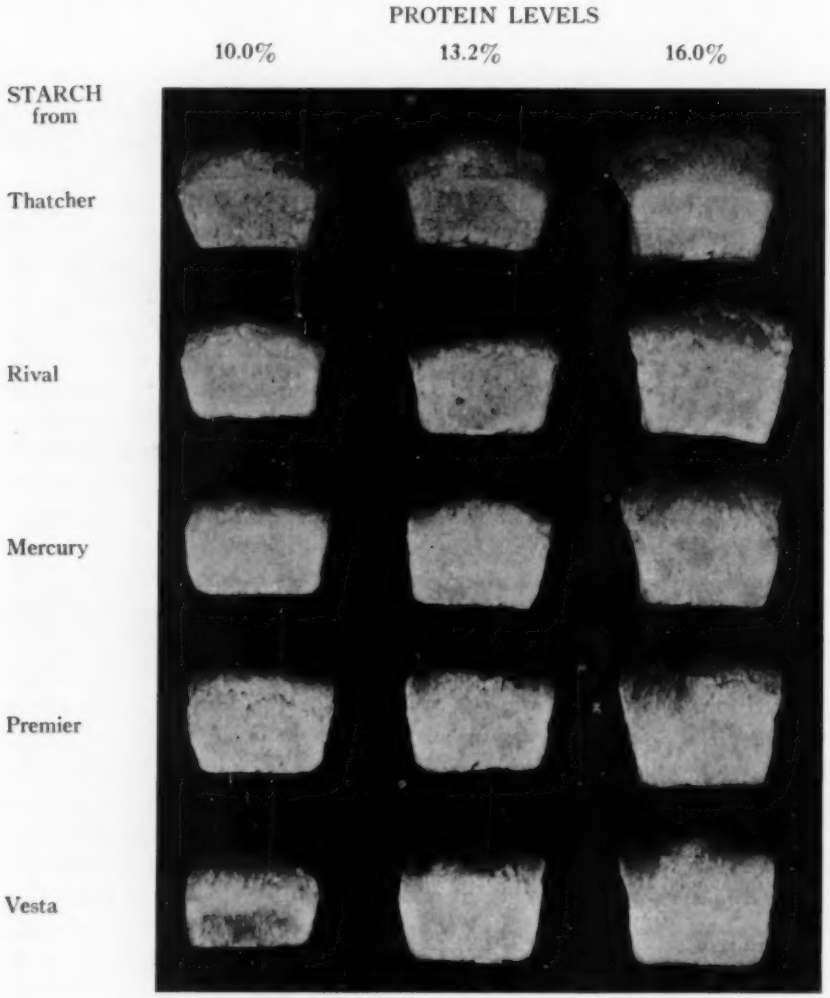

Fig. 1. Outward appearance of loaves baked from blends of hard red spring wheat starch with a common gluten substrate.

and by 75 cc. at the 16.0% level. The loaves produced with starch from the soft red winter wheat were bold, with even symmetry and good crust color. The hard red winter blends, however, yielded loaves of poor volume and ragged appearance with a tendency toward flat

top at the lower protein levels. The 16.0% protein loaf was considerably better, but only equal to the loaf from the 10.0% protein soft red winter blend. The crust colors were also pale and dull in the loaves made with hard wheat starch. The texture and crust color increased

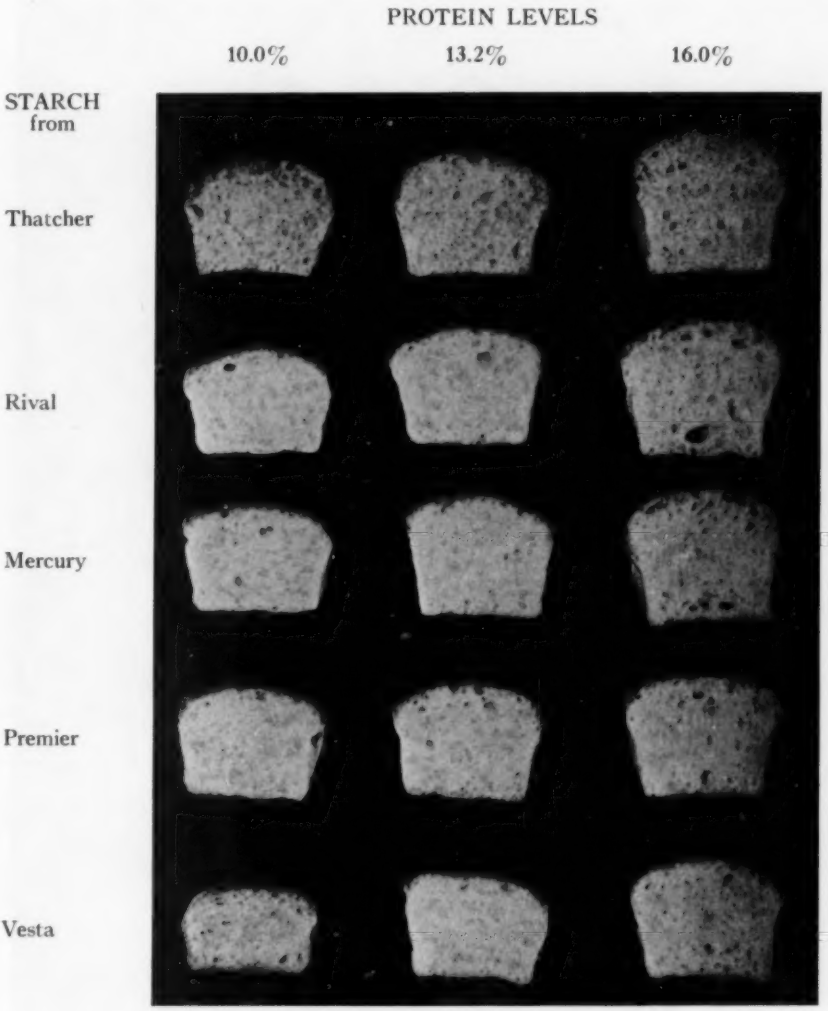

Fig. 2. Cut surfaces of loaves baked from blends of hard red spring wheat starch with a common gluten substrate.

with protein content in both wheat classes, but both of these loaf characters were decidedly better in the blends of soft red winter wheat starch.

In the comparison between the hard and soft white wheat, the

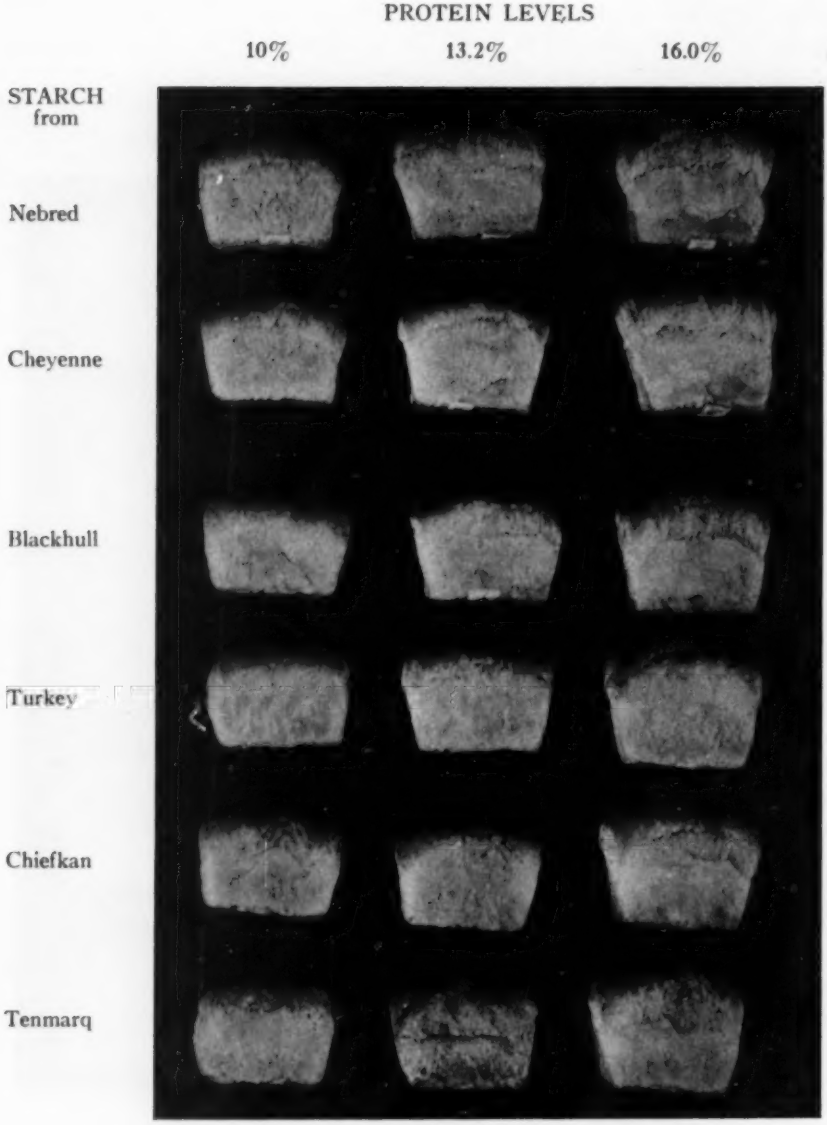

Fig. 3. Outward appearance of loaves baked from blends of hard red winter wheat starch with a common gluten substrate.

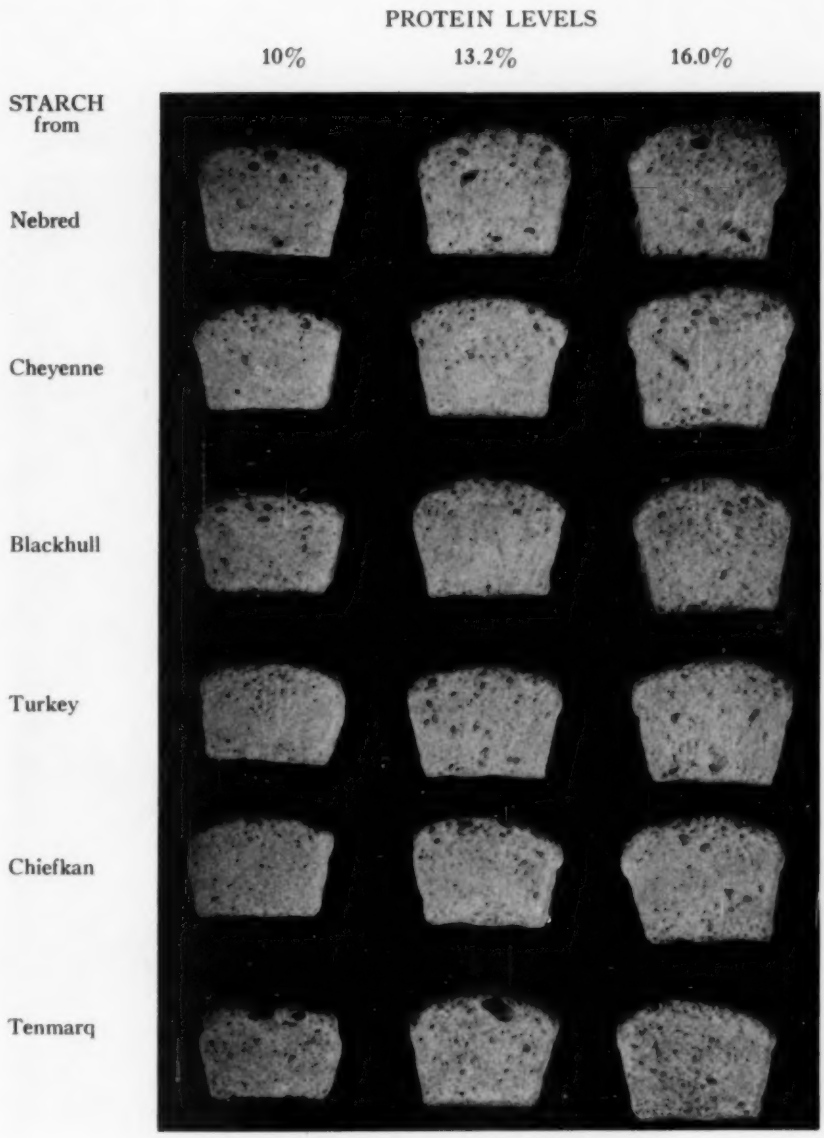

Fig. 4. Cut surfaces of loaves baked from blends of hard red winter wheat starch with a common gluten substrate.

soft-wheat starch again yielded better loaves than the hard-wheat starch at equivalent protein levels, but the difference between the two series of loaves was not so great as between the hard and soft red winter wheat starches.

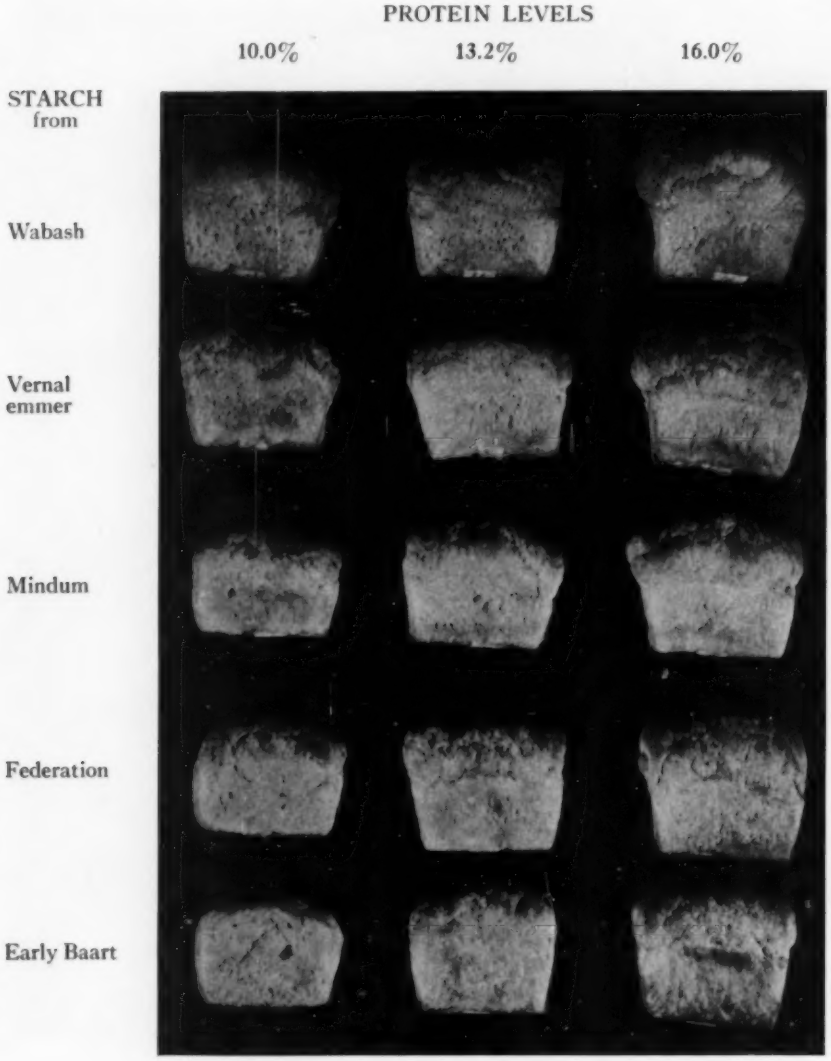

Fig. 5. Outward appearance of loaves baked from blends of different wheat starches with a common gluten substrate.

It would appear that these characteristic differences in baking quality between hard and soft starch blends might be attributed, in part at least, to a greater degree of starch damage during milling in the

case of the hard wheats, as pointed out by Sandstedt, Jolitz, and Blish (1939).

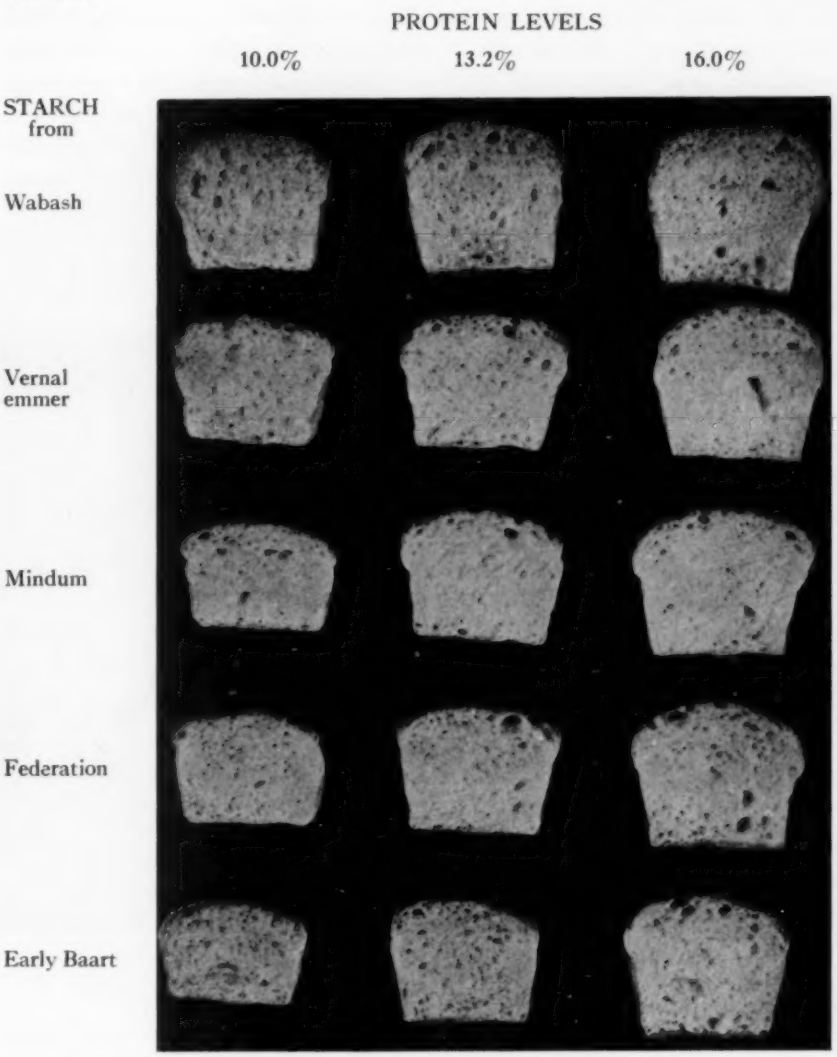

Fig. 6. Cut surfaces of loaves baked from blends of different wheat starches with a common gluten substrate.

Summary and Conclusions

The method of baking dry starch-gluten mixtures developed by Sandstedt, Jolitz, and Blish (1939) was applied to the determination of the baking quality of the starches prepared from a series of wheats embracing samples of the hard red spring, hard red winter, soft red

winter, durum, and white wheat classes. A constant gluten substrate prepared from hard red spring wheat was used in all the bakings. An initial period of one hour was allowed for the gluten to become hydrated before mixing with the starch. A mixing period of 2½ minutes was used and gave satisfactory results. Three protein levels were used for the mixes: 10.0%, 13.2%, and 16.0% on a 13.5% moisture basis. With these various protein levels, information regarding the differential reaction of the starch-gluten blends to different protein contents could

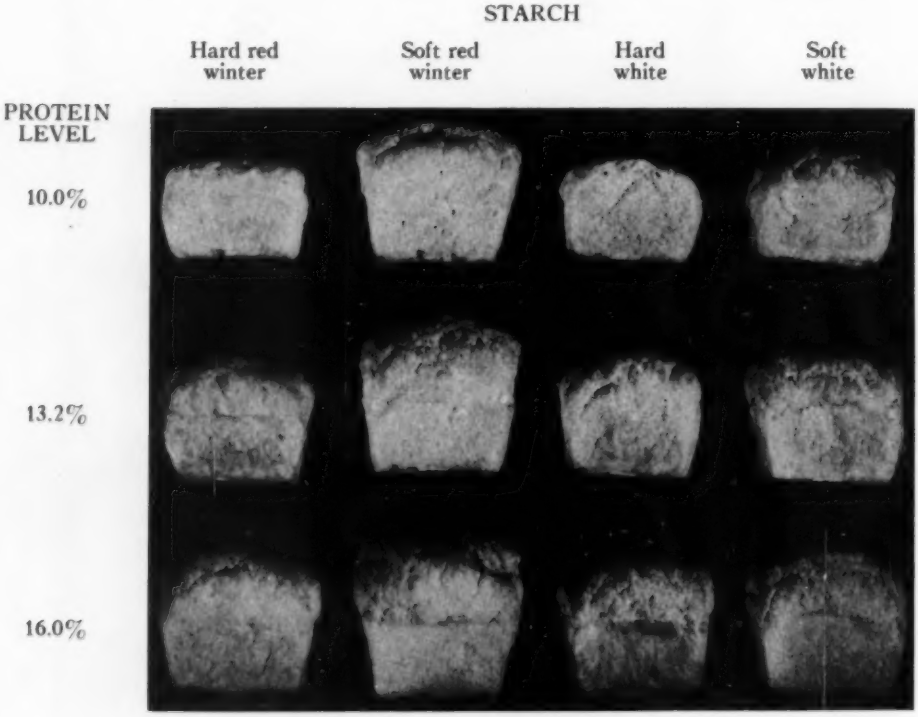

Fig. 7. Comparative loaves baked from blends of hard and soft winter and white wheat starches with a common gluten substrate.

be obtained. The phosphate-bromate baking method was employed, with 7% sucrose.

The baking results showed marked differences between the protein levels in terms of loaf volume, color, texture, and symmetry. The 16.0% protein blends produced good loaves approximating, in many instances, loaves baked from hard wheat flour of equivalent protein content. The absorption was raised in nearly every instance by increasing the percentage of gluten in the blend. Marked differences in loaf volume are shown by blends made from the different starches.

The starch prepared from a soft red winter wheat variety, Wabash, produced the best loaf in the series. Superior results in terms of loaf volume were also yielded by starches from a hard red spring wheat, Thatcher, and the hard red winter varieties Nebred and Cheyenne. The latter varieties were grown in 1939. A sample of Chiefkan grown in 1938 was superior to the 1939 sample of Chiefkan.

A sample of emmer starch gave satisfactory results. The white-wheat starches baked into loaves which were below average in loaf

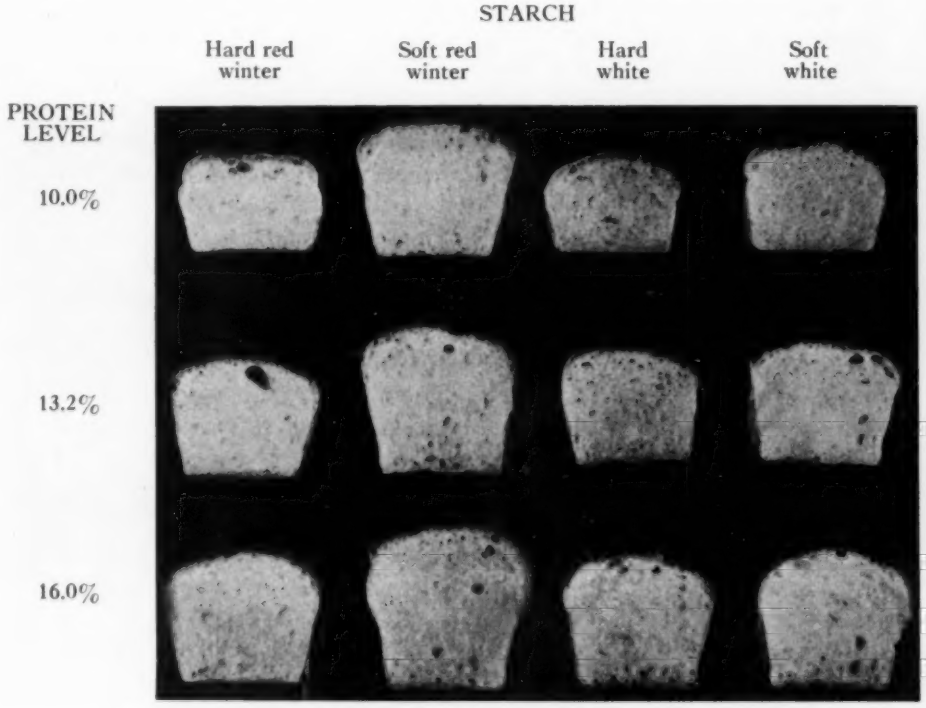

Fig. 8. Cut surfaces of loaves baked from blends of hard and soft winter and white wheat starches with a common gluten substrate.

volume. Turkey starch was also disappointing in its effect upon the loaf volume of the starch-gluten doughs, but the crumb color was satisfactory. Tenmarq starch, in like manner, was relatively low in loaf volume and symmetry at all protein levels.

It appears from the results that marked differences in those properties that influence baking quality are inherent in starches prepared as described from different wheat varieties. These differences may be related to injury to the starch granule during the operation of milling, with consequent effect upon the baking quality. The causes of such

dissimilarities are probably to a large degree heritable, but it is possible that environmental conditions are also involved. It is pointed out that in one instance where two samples of the same wheat grown in different years were examined considerable disparity in the loaves baked from the starch gluten blends was evident. Further investigations to answer these questions more fully are scheduled in the Department of Cereal Technology, North Dakota Experiment Station.

Acknowledgments

The authors wish to acknowledge the courtesy of the following in supplying wheat samples used in this investigation: Dr. John H. Parker, Prof. W. W. Worzella, Prof. R. M. Sandstedt, Mr. Art King.

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THE ACTION OF THE AMYLASES OF TWO BARLEY VARIETIES ON THE BARLEY STARCHES

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(Read at the Annual Meeting, May 1940)

The malts made from the two varieties of barley, Oderbrucker C.I. No. 4666 (Wisconsin Pedigree 5–1) and Wisconsin Barbless C.I. No. 5105 (Wisconsin Pedigree 38), differ decidedly in certain characters determined by malt analysis (Dickson *et al.*, 1938). Wisconsin Barbless appears to be deficient in the various enzyme systems as determined by diastatic power, yield of extract, and the amount of nitrogen made soluble during the mashing process. The two varieties do not differ greatly in total quantity of starch, nitrogen, and pentosans in either barley or malt.

A great deal of work has been done on the amylases of barley and malt, the results of which have been reviewed by Hanes (1937). In these studies the substrates used have been primarily potato starch, although Stamberg and Bailey (1938, 1939) and Blish, Sandstedt, and Mecham (1937) have recently studied the action of the amylases of wheat on wheat starches. Very little information is available on the action of the enzymes on barley starches. Baker and Hulton (1938) found that the same hydrolytic products were obtained from the action of malt amylases on malt starch as on potato starch.

As a phase of the study of the factors constituting quality in malt, it seemed desirable to investigate the activity of the isolated alpha- and beta-amylases from two varieties of barley on substrates of their own starches.

Materials and Methods

The barleys used in this investigation, Oderbrucker and Wisconsin Barbless, were grown at Urbana, Illinois, in 1938. Malts were produced from these barleys in the experimental malting unit in the Regional Malting Laboratory as a part of the regional series for that year.

The methods described by van Klinkenberg as modified by Hanes (1935) were used in the isolation of the two amylases. The beta-amylase was extracted from the ungerminated barleys which had been pearled to remove the hulls, aleurone layer, and most of the germ tissue and then ground to a fine flour in a Wiley mill. A Sharples supercentri-

[1] Cooperative investigations between the Division of Cereal Crops and Diseases, Bureau of Plant Industry, U. S. Department of Agriculture, and the University of Wisconsin, Madison, Wisconsin.

fuge was used to separate rapidly the precipitated enzyme from the alcohol solutions.

The alpha-amylases were prepared from the malts after the hulls had been removed and the remaining portion ground to a fine flour. A water suspension of the alcohol precipitate was heated to 70° C. for 15 minutes to inactivate the beta-amylase present, followed by precipitation with 65% alcohol, centrifuging, and drying. The Wigsman diffusion test and the Wohlgemuth method showed the enzymes to be relatively pure preparations.

The starches from the two varieties were isolated by a wet grinding process applied to the steeped, pearled barleys. The starch was passed through a 200-mesh sieve and the residue reground several times to increase the yield. The resulting starches were treated with 0.1% NaOH overnight at room temperature, washed several times by decantation, and finally collected and washed in a basket centrifuge. After a preliminary short drying in a vacuum oven, drying was completed in an air oven at 50°C. The yields of starch varied between 50% and 55% of the pearled barley.

Portions of each starch preparation were modified by the Lintner method with 7.5% hydrochloric acid at room temperature for 7 days, the acid being renewed every two days. In the following discussion these shall be referred to as modified starches. The analyses of the raw and modified starches are given in Table I.

TABLE I

CHARACTERISTICS OF THE STARCHES USED AS SUBSTRATES

Type of starch	Moisture	Total nitrogen	Total ash	Reducing power as theoretical maltose
	%	%	%	%
Raw—Oderbrucker	5.98	0.060	0.15	0.17
Soluble—Oderbrucker	7.30	0.035	0.30	3.56
Raw—Wisconsin Barbless	4.89	0.280	0.12	0.93
Soluble—Wisconsin Barbless	6.16	0.190	0.09	3.68
Soluble—Merck's Potato	11.92	0.014	0.26	2.28

The modified barley starches and Merck's soluble potato starch were used in 2% concentration, while the raw starches were used in 1% concentration. The untreated raw starches were triturated with several changes of distilled water, made to volume and suspended as well as possible by frequent shaking. The other starch solutions were prepared by adding the proper quantity of starch to boiling water and boiling for two minutes in order to disperse them. The solutions to be used with

beta-amylase were adjusted to pH 4.7 and those with alpha-amylase to pH 5.4 by appropriate acetic acid-sodium acetate mixtures.

All hydrolyses were carried out in cork-stoppered flasks in a water bath at 40°C. in the presence of a few drops of toluene, using 100 ml. of starch solution and 10 ml. of enzyme solution. In order to determine the reaction rates, a procedure similar to that used by Redfern and Johnston (1938) was used, aliquots being withdrawn at regular, short intervals until 30 minutes had elapsed from the time of addition of enzyme to the starch solution. The aliquots were immediately transferred to flasks containing alkaline ferricyanide reagent, and the reducing power determined by the method of Anderson and Sallans (1937). The reaction rates were determined from the slope of curves obtained by plotting milligrams of maltose produced against reaction time in minutes, and these were calculated for equal enzyme concentrations from the two varieties as milligrams of maltose produced per minute.

After five days the final reducing powers of the digests were determined and these were expressed as the percentage of the theoretical amount of maltose that could be obtained from the quantity of starch.

Presentation and Discussion of Data

The enzyme isolation procedures for the two varieties were made as nearly identical as possible in order to determine comparative yields. The respective yields of beta-amylase from Oderbrucker and Wisconsin Barbless barleys were 1.42 and 1.18 g. from 500 g. of barley flour, which was equivalent to 0.26% and 0.22% of the dry weight of barley flour. The yields of alpha-amylase from the malts from the same two barleys were 0.65 and 0.34 g. or 0.14% and 0.07% of the dry weight of the malts, respectively. The Oderbrucker variety yielded somewhat larger quantities of both enzymes than the Wisconsin Barbless.

Typical reaction-rate curves for alpha- and beta-amylase preparations from the Oderbrucker and Wisconsin Barbless barleys acting on the Oderbrucker soluble starch are shown in Figure 1. From an inspection of this figure, it is apparent that the activities of the alpha-amylases from the two varieties were not greatly different, while the beta-amylase from the Oderbrucker barley was more than twice as active as that from the Wisconsin Barbless. A summary of the reaction rates and hydrolysis limits of the four enzyme preparations upon the seven substrates is given in Table II. These values represent means of two or more duplicate runs. The data for reaction rates and hydrolysis limits, respectively, are illustrated by bar diagrams in Figures 2 and 3. No values are given for the reaction rates of either enzyme on the untreated raw starches

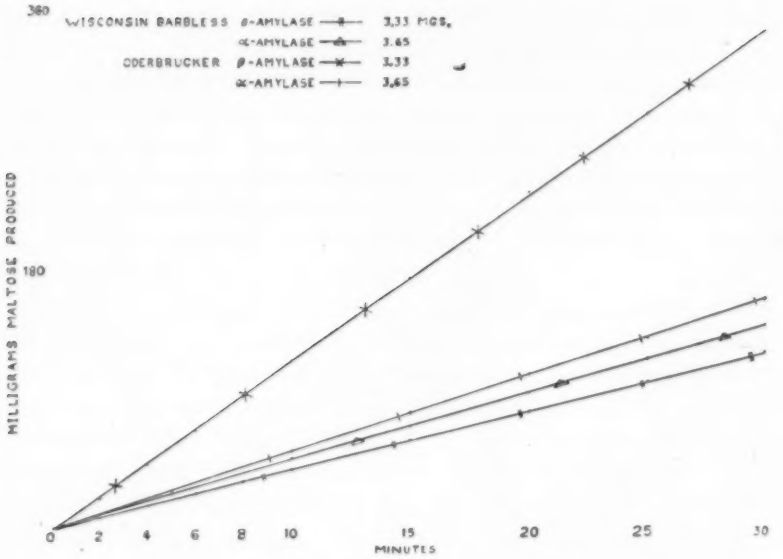

Fig. 1. Typical reaction rate curves for the action of the amylases on soluble Oderbrucker starch.

TABLE II

SUMMARY OF REACTION RATES AND HYDROLYSIS LIMITS OF THE ALPHA- AND BETA-AMYLASES ACTING UPON THE VARIOUS SUBSTRATES (MEANS OF TWO OR MORE DUPLICATE RUNS)

Alpha-amylase 3.65 mg. per 110 ml.
Beta-amylase 3.33 mg. per 110 ml.

Variety and amylase	Activity	Type of substrate						
		Soluble potato	Soluble Oderbrucker	Soluble Wis. Barbless	Boiled raw Oderbrucker	Boiled raw Wis. Barbless	Raw Oderbrucker	Raw Wis. Barbless
Oderbrucker—Alpha	*RR* [1]	5.6	5.1	5.2	4.9	4.9	—	—
	HL [2]	55.0	53.4	56.9	51.3	50.4	2.5	15.3
Wis. Barbless—Alpha	*RR*	5.1	4.6	4.8	4.4	4.4	—	—
	HL	54.9	52.8	55.3	50.6	47.6	2.8	14.2
Oderbrucker—Beta	*RR*	11.0	11.6	9.7	7.3	5.7	—	—
	HL	61.8	62.8	53.9	48.5	53.6	1.2	2.9
Wis. Barbless—Beta	*RR*	3.9	4.0	3.7	2.0	1.9	—	—
	HL	60.6	63.3	56.4	45.8	50.4	1.3	3.1
Oderbrucker—Alpha + Beta	*HL*	—	—	—	—	—	4.5	18.4
Wis. Barbless—Alpha + Beta	*HL*	—	—	—	—	—	4.0	19.4

[1] *RR* indicates reaction rate in milligrams of maltose per minute.
[2] *HL* indicates hydrolysis limit after 120 hours as percentage of complete theoretical hydrolysis.

since the rates were so low that a satisfactory measurement could not be made.

From an inspection of Table II and Figure 2, it is evident that the alpha-amylase from the Wisconsin Barbless variety showed a lower activity on all substrates than that from the Oderbrucker, but the differences are not large. Considering the various substrates, the highest activity of the alpha-amylases was obtained on the soluble potato starch.

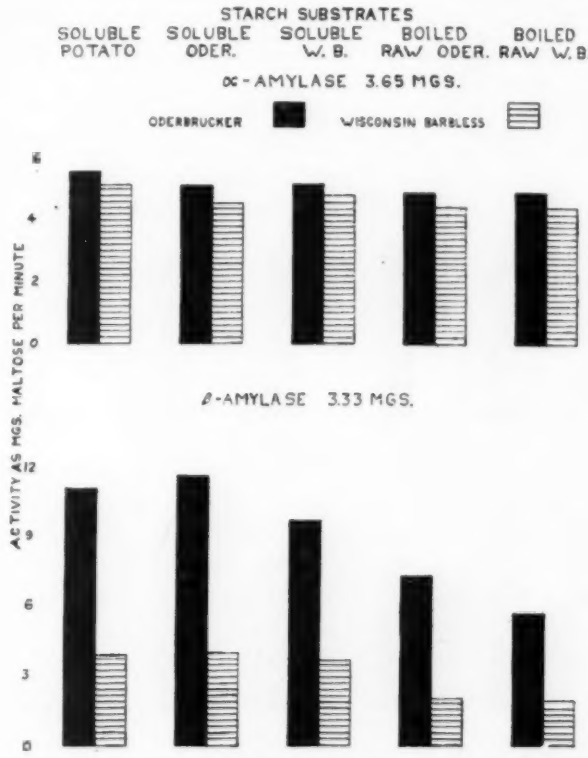

Fig. 2. Reaction rates, in milligrams of maltose per minute, of the alpha- and beta-amylases from the two varieties on five starch substrates.

The rate of action on the boiled raw barley starches was only slightly less than on the modified barley starches. Differences in the susceptibility of the starches from the two varieties were not readily apparent when only the activity of the alpha-amylases was considered, although there was a slight indication that the alpha-amylase from Wisconsin Barbless was somewhat more active on the modified starch from that variety than on the Oderbrucker starch.

The enormous difference in rate between the beta-amylases from the two varieties is shown in Table II and Figure 2. This difference was

much greater than would be anticipated from the diastatic powers of the two barleys based on the water extractions and the papain digestions. The values determined on the barleys were: for Oderbrucker, water extraction 66°L., papain digestion 125°L.; for Wisconsin Barbless, water extraction 56°L., papain digestion 122°L. The activity measurements were made at 40°C., while the diastatic powers were determined at the usual temperature of 20°C. It seems unlikely that the difference could be caused by a differential effect of temperature on the enzyme activities. Although as nearly identical procedures as possible were used for isolation, there is always the possibility of differences in solubility

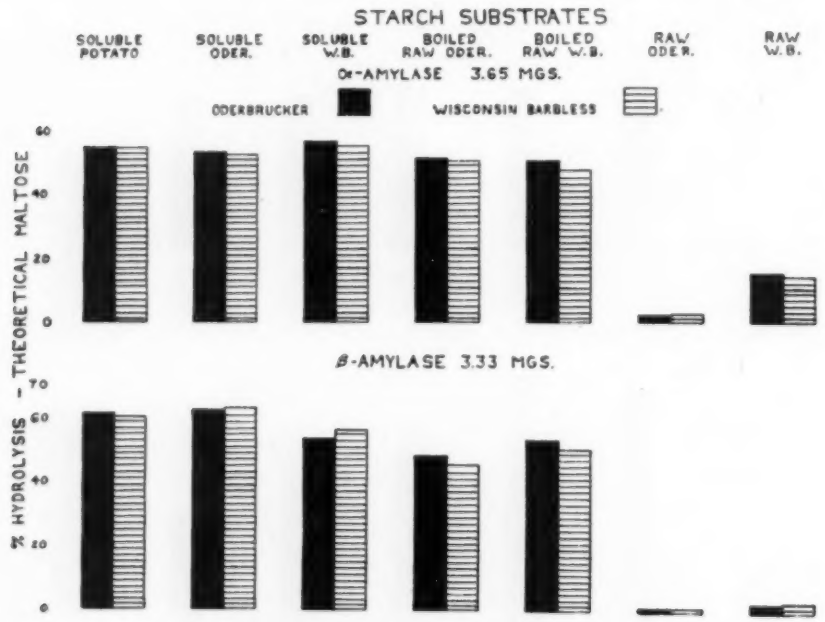

Fig. 3. Hydrolysis limits as percentage theoretical maltose after 120 hours, produced by the alpha- and beta-amylases from the two varieties acting upon seven substrates.

of the enzymes from the two barleys as well as the much debated question of the presence or absence of activators or inhibitors.

The Wisconsin Barbless soluble and boiled raw starches appeared to be more resistant to the action of the beta-amylase from the Oderbrucker variety than the corresponding Oderbrucker starches. The same trend was apparent with the Wisconsin Barbless beta-amylase, although the differences were small and probably not significant. As would be expected, the modified or soluble starches were much more susceptible to the action of beta-amylases than the boiled raw starches in contrast to the action of the alpha-amylases where the differences were slight.

The hydrolysis limits in percentage of the theoretical maltose are given in Table II and Figure 3 for the four enzymes acting upon the seven substrates for 120 hours. Considering the alpha-amylases, the values varied between 50% and 57% hydrolysis for the modified and boiled starches and the variations due to source of enzyme and substrate were not great. A slightly higher limit was reached on the soluble Wisconsin Barbless starch than on the soluble Oderbrucker by the alpha-amylase from both varieties, which agrees with the results obtained on the reaction rates. Here again the hydrolysis limits obtained on the boiled raw starches were only slightly below those on the modified starches.

As noted earlier, the reaction rates of the Oderbrucker beta-amylase were much greater than the Wisconsin Barbless, but differences in hydrolysis limits were much smaller. In fact, on the modified starches from both varieties, the beta-amylase from Wisconsin Barbless hydrolyzed slightly more starch than that from Oderbrucker, but the difference was not significant. Considering the various substrates, the modified Wisconsin Barbless starch appeared to be somewhat more resistant to beta-amylase hydrolysis than the modified Oderbrucker starch. With the boiled raw starches from the two varieties the reverse seemed to be true. The hydrolysis limits obtained from the action of the beta-amylases on the soluble potato and soluble Oderbrucker barley starch were in the usual range reported in the literature (62%–67%) (Hanes, 1937). However, the final limits on the soluble Wisconsin Barbless starch and the boiled raw starches from both varieties were significantly reduced.

Several anomalies were evident. For example, the Wisconsin Barbless modified starch appeared to be more susceptible to the action of alpha-amylase and less susceptible to the action of beta-amylase than the corresponding Oderbrucker starch. There was some evidence that the reverse situation existed with the boiled raw starches. On the basis of the limits of hydrolysis by beta-amylase, the boiled raw Oderbrucker starch was appreciably more resistant to enzyme action than the modified Oderbrucker starch, but the corresponding Wisconsin Barbless starches did not show such a difference.

The action of the beta-amylase on suspended raw barley starch was very slight. The starch from Wisconsin Barbless was hydrolyzed more than twice as much as that from Oderbrucker. Likewise the Wisconsin Barbless starch was more than five times as susceptible to alpha-amylase. These facts, combined with the higher reducing power of this starch as shown in Table I, might indicate a larger percentage of broken granules in this sample as an explanation for its greater susceptibility. Microscopical examination did not show this to be the case, although there were

many more small granules in the Wisconsin Barbless starch than in the Oderbrucker. The combined action of alpha- and beta-amylases on the raw starches was roughly equal to the sum of the individual actions of the two enzymes. These values were obtained on suspensions that were not shaken continuously, and, as pointed out by Blish, Sandstedt, and Mecham (1937) accurate determinations of enzyme action are difficult under such conditions.

The combined action of the two amylases from Oderbrucker on the modified and boiled raw starches was investigated further. In one case the alpha- and beta-amylases were allowed to act simultaneously; in the other, the alpha-amylase was allowed to act for 60 hours, then the beta-amylase was added and both allowed to act for an additional 60 hours. The results are given in Table III in which the bottom line represents

TABLE III

RESULTS ON THE COMBINED ACTION OF ODERBRUCKER ALPHA- AND BETA-AMYLASES ON THE MODIFIED AND BOILED RAW STARCHES

Alpha-amylase 3.65 mg. per 110 ml.
Beta-amylase 3.33 mg. per 110 ml.

Combination of amylases	Activity	Type of substrate				
		Soluble potato	Soluble Oderbrucker	Soluble Wis. Barbless	Boiled raw Oderbrucker	Boiled raw Wis. Barbless
Alpha- and beta-amylases simultaneously	*RR* [1]	13.4	15.5	14.2	13.8	12.2
	HL [2]	76.5	82.3	74.4	84.5	71.3
Alpha-amylase followed by beta-amylase	*HL*	78.7	70.8	65.0	82.2	75.8
Sum of individual alpha- and beta-amylases	*RR*	16.6	16.7	14.9	12.2	10.6

[1] *RR* indicates reaction rate in milligrams of maltose per minute.
[2] *HL* indicates hydrolysis limit after 120 hours as percentage of complete theoretical hydrolysis.

the sum of the individual reaction rates for the two enzymes as given in Table II. As would be expected, the two enzymes acting together show a definite increase in activity over the individual action of either one. The combined action of the amylases on the soluble starches was not so great as the sum of the individual actions of alpha- and beta-amylase, but on the boiled raw starches the reverse was true. This difference is probably explained by the fact that the boiled raw starches were almost as susceptible to alpha-amylase action as the soluble starches, but were considerably more resistant to beta-amylase. However, when the enzymes acted together, the dextrin fragments from the action of alpha-amylase were rapidly broken down by the beta-amylase. Alpha-amylase followed by beta-amylase gave a lower hydrolysis limit than the combined

action on the soluble barley starches, but on the boiled raw starches the differences were not great. In all cases where combinations of the two enzymes were used, the soluble and boiled raw Oderbrucker starches were more susceptible to hydrolysis than the corresponding Wisconsin Barbless starches.

A limited study was made on the influence of enzyme concentration on the hydrolysis limit, using a twofold increase of beta-amylase and two- and fourfold increases of alpha-amylase. The data are presented in Table IV. With the soluble potato and soluble Oderbrucker starch,

TABLE IV

THE INFLUENCE OF CONCENTRATION OF ALPHA- AND BETA-AMYLASES ON THE HYDROLYSIS LIMIT AFTER 120 HOURS OBTAINED ON THE VARIOUS SUBSTRATES

Enzyme	Enzyme concentration, mg. per 100 ml.	Type of substrate				
		Soluble potato	Soluble Oderbrucker	Soluble Wis. Barbless	Boiled raw Oderbrucker	Boiled raw Wis. Barbless
		HL[1]	*HL*[1]	*HL*[1]	*HL*[1]	*HL*[1]
Oderbrucker—Beta	3.33	61.8	62.8	53.9	48.5	53.6
	6.66	61.2	62.7	61.1	56.8	55.0
Wis. Barbless—Beta	3.33	60.6	63.3	56.4	45.8	50.4
	6.66	60.1	61.4	56.8	52.7	52.2
Oderbrucker—Alpha	3.65	55.0	53.4	56.9	51.3	50.4
	7.30	58.0	59.1	62.6	54.2	56.2
	14.60	64.7	71.7	75.4	63.2	64.9
Wis. Barbless—Alpha	3.65	54.9	52.8	55.3	50.6	47.6
	7.30	57.0	58.3	61.5	55.5	55.8
	14.60	63.4	67.3	72.7	69.6	62.8

[1] *HL* is the hydrolysis limit.

increasing the concentration of beta-amylase did not affect the hydrolysis limits and the values obtained agreed satisfactorily with those obtained by Hanes (1935). With the soluble Wisconsin Barbless starch, doubling the enzyme concentration increased the hydrolysis limit and gave a value which was in agreement with the other two soluble starches. In the case of the boiled raw starches, a greater enzyme concentration increased the hydrolysis limit, but the value was still appreciably below the soluble starch values.

It is seen that the hydrolysis limits obtained with alpha-amylase are dependent upon the enzyme concentration as shown by Hanes (1935). The limits of hydrolysis obtained were much higher than those generally obtained, although values of similar magnitude have been reported as summarized by Hanes (1937). This could be explained by contamina-

tion of the alpha-amylase by beta, although, as previously stated, both enzymes appeared to be relatively pure. Another possibility is that with the relatively long reaction time used, appreciable quantities of glucose may be formed which would increase the reducing power of the digest. These data substantiate those in Table II in showing that soluble Wisconsin Barbless starch is more susceptible to the action of alpha-amylase and less susceptible to the action of beta-amylase than that from Oderbrucker.

Several interesting differences in the starches as substrates have been indicated by this study, and further work is in progress in an attempt to explain them.

Summary

Preparations of alpha- and beta-amylases were made from Oderbrucker and Wisconsin Barbless barleys and malts. The starches were isolated from the same two barleys and portions modified in various ways.

The action of the amylases on the isolated starches was studied, reaction rates and final saccharification limits being determined.

Oderbrucker yielded 100% more alpha-amylase and 15% more beta-amylase than Wisconsin Barbless. The activities of the isolated alpha-amylases from the two varieties were about the same, but the beta-amylase from Oderbrucker was approximately 2½ times as active as that from Wisconsin Barbless.

Although the evidence is not entirely conclusive, there were indications that the modified and boiled raw Oderbrucker starches were more susceptible to beta-amylase degradation than the Wisconsin Barbless starches. However, the Wisconsin Barbless modified and raw starches were more susceptible to alpha-amylase action than the Oderbrucker starches.

Studies with combinations of the two enzymes, and the individual enzymes in different concentrations, essentially substantiated the above conclusions.

Acknowledgments

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FACTORS WHICH INFLUENCE THE PHYSICAL PROPERTIES OF DOUGH.[1] III. EFFECT OF PROTEIN CONTENT AND ABSORPTION ON THE PATTERN OF CURVES MADE ON THE RECORDING DOUGH MIXER

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Experience with a large number of curves made on the recording dough mixer has shown that the amounts of protein and of water used in mixing the dough influence the pattern, particularly the height of the curves. The illustrations presented in this paper will bring this out more fully. The wide variations that may occur in curves were shown by Swanson (1939). No attempt was made in that paper to relate these variations to protein content. Curves with little or no rise in the first part are generally from low-protein flours, while curves with a steep rise and good height are from high-protein flours. Variety also has an influence. Thus flours from Chiefkan and Clarkan, which belong to the Blackhull group, usually give curves which have a steeper rise and somewhat greater height than flours of the same protein content from Turkey and Tenmarq. Also, when the protein content of a hard red winter wheat is low the curves become similar to those obtained from soft red winter wheats (Larmour, Working, and Ofelt, 1939, 1940).

[1] Contribution No. 72, Department of Milling Industry.

The purpose of this paper is to show how the curve pattern may be influenced by the protein content of the flours, whether this is the amount that naturally occurs in the flour or whether it has been modified by blending with starch or with a low-protein flour. It will also be shown that the percentage of absorption is an important factor especially in determining the height of the curves.

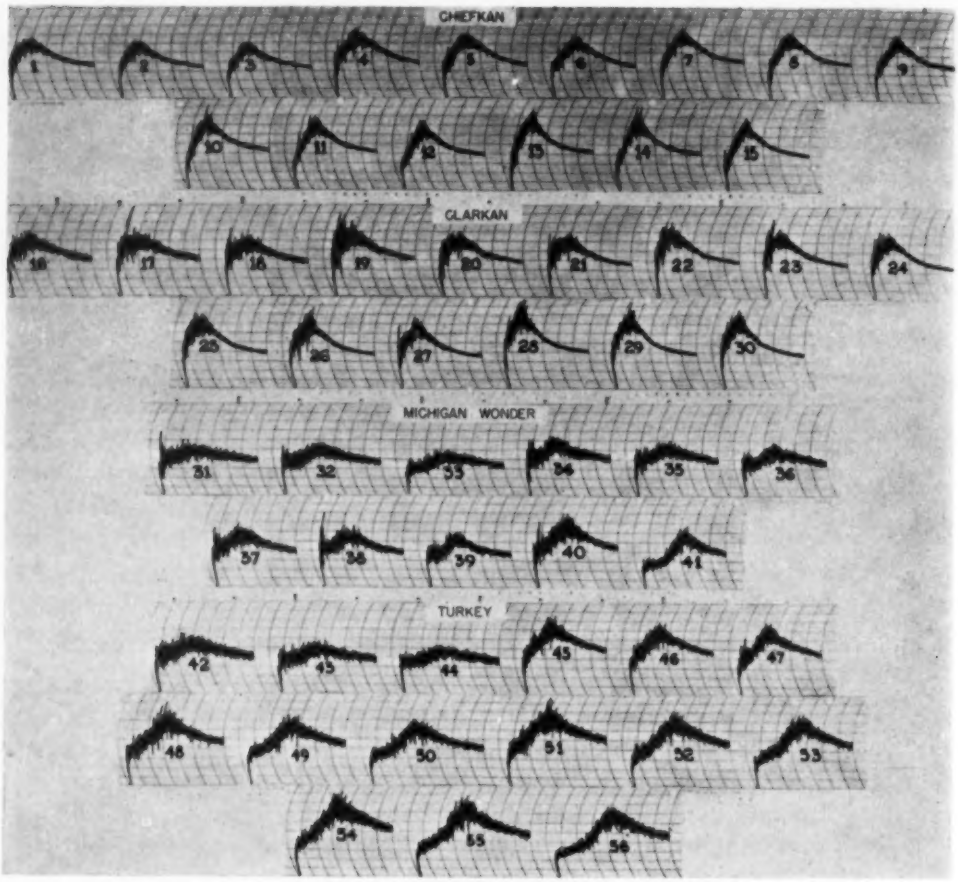

Fig. 1. Effects of variations in protein content and of absorption in flours from four varieties of wheat. (See Table I.)

Figure 1 shows the effects on curve patterns of variations in protein content and of absorption in flours from four varieties. These flours were milled from wheat varieties grown in co-operative experiments conducted in various parts of the state by A. L. Clapp of the Department of Agronomy. The wheat samples from various localities were blended so as to obtain samples at various protein levels.

In Table I are found the identification numbers for each of the curves, together with absorptions used in making them and also the protein contents of the flours used. The curves of each variety are arranged in groups of three, and the groups are arranged in ascending order of protein content. The first group to the left for each variety has the lowest protein, and the last group the highest protein. The middle curve in each group was made with the optimum absorption as determined on the flour-water dough. The first curve in each group was made with 2% less water and the third curve with 2% more water than the optimum. The amount of water needed to make a dough of

TABLE I

THE PROTEIN CONTENT AND ABSORPITON OF FLOURS OF FOUR VARIETIES USED TO MAKE THE CURVES IN FIGURE 1

Variety	Sample No.	Protein	Absorptions			Curve Nos., Fig. 1		
		%	%	%	%			
Chiefkan	25042	9.9	58.0	60.0	62.0	1	2	3
	25043	11.2	60.5	62.5	64.5	4	5	6
	25044	12.7	63.5	65.5	67.5	7	8	9
	25045	14.1	67.5	69.5	71.5	10	11	12
	25046	15.4	68.5	70.5	72.5	13	14	15
Clarkan	25056	9.9	51.0	53.0	55.0	16	17	18
	25057	10.6	52.0	54.0	56.0	19	20	21
	25058	12.2	55.0	57.0	59.0	22	23	24
	25059	14.0	59.5	61.5	63.5	25	26	27
	25060	15.2	62.0	64.0	66.0	28	29	30
Michigan Wonder	25079	9.7	52.0	54.0	56.0	31	32	33
	25080	10.6	53.5	55.5	57.5	34	35	36
	25081	12.5	55.5	57.5	59.5	37	38	39
	25082	14.3	63.0	—	67.0	40	—	41
Turkey	25096	9.5	55.0	57.0	59.0	42	43	44
	25097	10.8	62.0	64.0	66.0	45	46	47
	25098	12.7	62.0	64.0	66.0	48	49	50
	25099	13.8	63.5	65.5	67.5	51	52	53
	25100	15.6	67.5	69.5	71.5	54	55	56

optimum consistency was determined by mixing flour and water in a micro mixer and " feeling " the dough. This method leaves much to be desired, since the results depend so much on the experience and judgment of the operator. However, it was the only practical method available for these experiments.

The curves in Figure 1 show that variations of 2% above or below the optimum absorption do not change the general " varietal " pattern except in height. An absorption below optimum will give a greater height than an absorption above the optimum. With some varieties such as Turkey an absorption above the optimum will have a tendency toward

production of a concave upslope. This tendency is not shown in the curves from Chiefkan and Clarkan, in which the upslope is convex. This concave upslope is also shown when the wheat has been damaged by high moisture (Swanson, 1941).

Thus the amount or percentage of absorption will influence the height, and in some flours the character of the upslope, but will not within reasonable limits modify the main pattern of the curves. The protein content is the more potent factor in affecting the height of the curves.

The varietal factors have the greatest influence on the curve pattern (Swanson, 1938). Thus in Figure 1 it is evident that Chiefkan has patterns very different from the others. As will be shown later, the patterns due to varietal characteristics are less pronounced in curves from low- than in curves from high-protein flours. That the protein content influences the height has also been shown by Larmour, Working, and Ofelt (1939). The height, however, is much less influenced by protein content in Chiefkan and Clarkan than in Michigan Wonder and Turkey.

Effect of Reducing the Protein Content to 10% by Adding Starch

The same flours of the four varieties used for making the curves shown in Figure 1 were diluted with wheat starch [2] so that all the mixtures had 10% protein. The curves obtained from Chiefkan, Clarkan, Michigan Wonder, and Turkey are shown in rows 1, 2, 3, and 4 respectively of Figure 2.

The legend for Figure 2 identifies each curve by number and gives the protein percentages of the original flours and the absorptions used. The first curve in each one of the four rows was made from the undiluted flour, since the protein content was a little below 10% as shown in Table I. For Chiefkan and Clarkan, in which the protein was very near 10%, the first curves show very little difference from those that were diluted. The curves made from the undiluted flour from Michigan Wonder and Turkey show notable differences from those that were made from the flours diluted to 10%.

In the fifth row of Figure 2 are shown curves that bring out the contrast between those made from Tenmarq (protein 12.8%), Turkey (protein 13.1%), and Chiefkan (protein 12.8%), both on the original flours and after dilution to 10% protein.

A comparison of curves in Figures 1 and 2 will show that while protein content is the greatest factor in influencing the pattern of the curves, the curve differences within a variety largely disappear when

[2] This was furnished by the Huron Milling Company, Huron, Michigan.

the protein content is reduced to the same level. Thus the curves from the flours in the range of 14% to 15% protein are similar to those that had been diluted from only a little above 10%. The main effect of dilution with starch is to reduce the height, but the varietal charac-

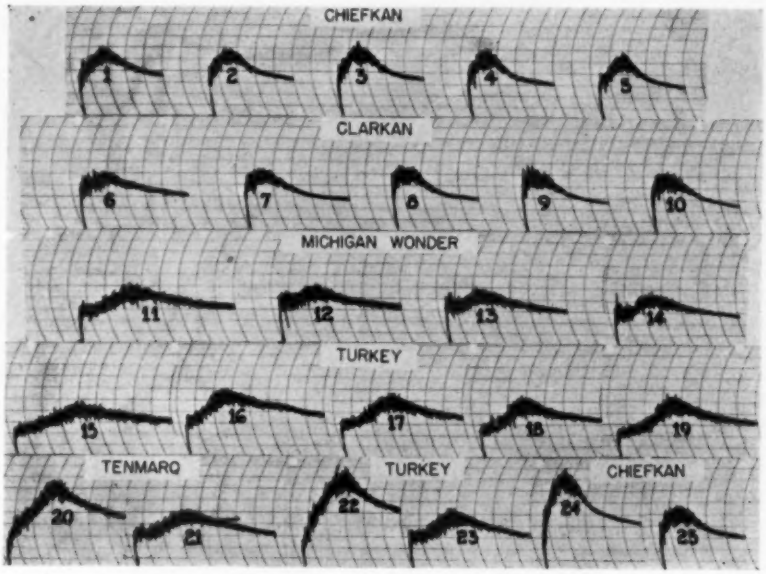

Fig. 2. Protein reduced to 10% by addition of starch.

	CHIEFKAN				
No. of curve	1	2	3	4	5
Original protein, %	9.9	11.2	12.7	14.1	15.4
Absorptions, %	62	64	65	66	67
	CLARKAN				
No. of curve	6	7	8	9	10
Original protein, %	9.9	10.6	12.2	14.0	15.2
Absorptions, %	62	62	62	62	61
	MICHIGAN WONDER				
No. of curve	11	12	13	14	
Original protein, %	9.7	10.6	12.5	14.3	
Absorptions, %	60	61	62	61	
	TURKEY				
No. of curve	15	16	17	18	19
Original protein, %	9.5	10.8	12.7	13.8	15.6
Absorptions, %	64	67	67	68	68

	TENMARQ		TURKEY		CHIEFKAN	
No. of curve	20	21	22	23	24	25
Original protein, %	12.8	—	13.1	—	12.8	—
Absorptions, %	68	68	64	66	62	65

teristics still persist. Thus if the protein content is made constant, all the curves from the flours of any one variety that differ in protein will be very similar. Whether these flours diluted with starch would have the same baking characteristics as 10% protein flours was not determined.

Effect of Progressive Dilution with Starch

Flours from Turkey (protein 13.1%), Tenmarq (protein 12.8%), and Chiefkan (protein 12.8%) were diluted progressively with the wheat starch used and also with ordinary edible corn starch. The proportions of flour and starch, and the calculated percentages of protein of the mixtures, are presented in Table II and the curves obtained from these mixtures are shown in Figure 3.

TABLE II

PROPORTIONS OF FLOUR AND STARCH MIXTURES AND PROTEIN PERCENTAGES

Proportions		Calculated protein content		
Flour	Starch	Turkey	Tenmarq	Chiefkan
g.	g.	%	%	%
35	0	13.10	12.80	12.80
30	5	11.19	10.98	10.97
25	10	9.32	9.15	9.14
20	15	7.45	7.32	7.31
15	20	5.59	5.49	5.48
10	25	3.73	3.66	3.66

The upper three rows are from dilution with wheat starch, and the lower three rows are from dilution with corn starch. The curves in Figure 3 show a progressive decrease in height with increasing starch ratios. The variety characteristics persist until the flour-starch ratio is about 20–15. The effect of corn starch is very different from that of wheat starch in the larger starch ratios. In these, the elastic characteristics have disappeared and the flour-starch-water mixture behaves more as a plastic mass. The same may be said of Chiefkan for the two larger wheat-starch ratios. The Turkey and Tenmarq mixtures with the larger amounts of wheat starch produce curves similar to those obtained from very soft, low-protein flours. The curves with the larger starch ratios make it appear that the flours of Tenmarq and Turkey in comparison with Chiefkan have longer gluten strands (Swanson, 1925, 1938), which maintain the dough characteristics when larger amounts of starch are present. In Chiefkan they seem to be shorter, and hence the plastic properties become evident with the smaller starch-flour ratios. There were some variations in absorptions of the different ratios of flour and starch, but these were not sufficient to have any material influence on the general patterns of the curves shown in Figure 3.

Effect of Blending a Low-Protein Flour with High-Protein Flours

A soft wheat flour of 8.3% protein was blended in various proportions with flours of Turkey, Tenmarq, and Chiefkan. The proportions

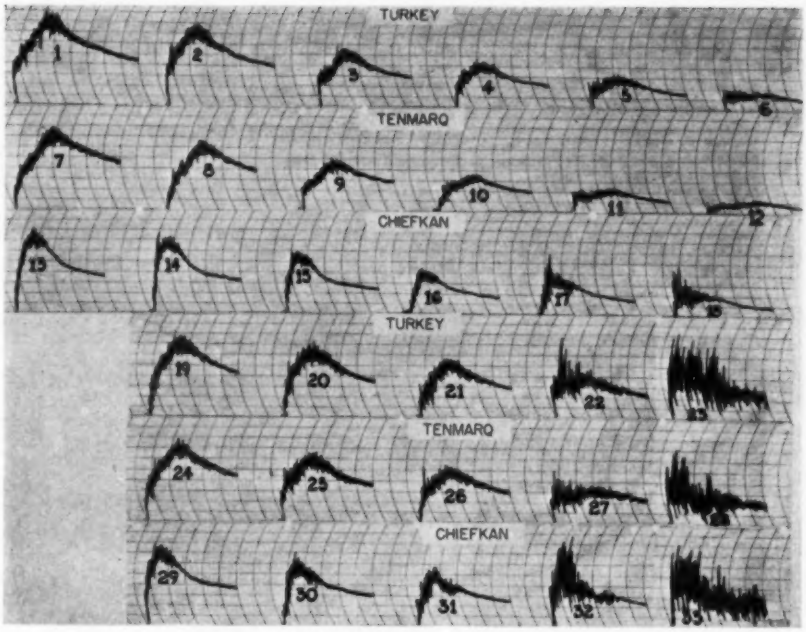

Fig. 3. Curves showing effect of progressive dilutions with starch.

	Ratio of Flour to Starch					
	35–0	30–5	25–10	20–15	15–20	10–25
	WHEAT STARCH (*Curve Numbers*)					
Turkey	1	2	3	4	5	6
Tenmarq	7	8	9	10	11	12
Chiefkan	13	14	15	16	17	18
	CORN STARCH (*Curve Numbers*)					
Turkey		19	20	21	22	23
Tenmarq		24	25	26	27	28
Chiefkan		29	30	31	32	33

of the blends, the calculated protein percentages of the blends, and the absorptions of the blends as well as the flours used in blending are given in Table III. The curves obtained on these blends are given in Figure 4. The first curve in each row is from all low-protein flour, and the last from all high-protein flour. The four intermediate are from the blends as indicated in Table III.

The curve from the low-protein flour used in blending reaches the maximum height in about a half minute. The upslope and the downslope are so short that they are almost obscured. At the end of the short downslope the curve becomes horizontal with a slow narrowing. The upward rise of the curves from the blends starts with the smaller proportion of the hard-wheat flours and becomes higher with the increasing amounts of these flours. The percentages for absorption increase, as would be expected, with the larger amounts of the high-protein

flours, the increases being greatest in Chiefkan and least in Turkey. The gradual change in the characteristics of these curves with increasing amounts of the high-protein flours indicates that the absorption percentages were suitable to these mixtures.

TABLE III

BLENDS OF LOW-PROTEIN FLOUR WITH HIGH-PROTEIN FLOURS

Proportions in blend		Calculated percentages protein in blends with			Absorptions of blends with		
Low protein	High protein	Turkey	Tenmarq	Chiefkan	Turkey	Tenmarq	Chiefkan
		%	%	%	%	%	%
100	0	8.3[1]	8.3[1]	8.3[1]	57[1]	57[1]	57[1]
80	20	9.3	9.2	9.2	58	59	59
60	40	10.2	10.1	10.1	60	61	62
40	60	11.2	11.0	11.0	61	63	64
20	80	12.1	11.9	11.9	62	64	66
0	100	13.2	13.0	13.1	63	66	68

[1] Protein percent and absorption of the soft-wheat flour used in mixing with the high-protein flours.

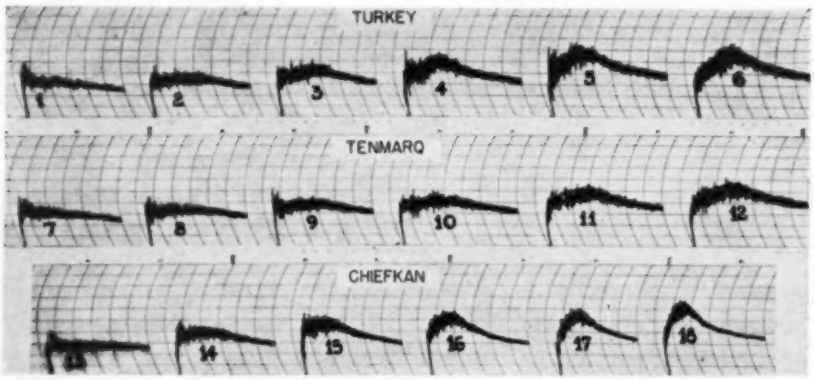

Fig. 4. Curves showing effect of blending a low-protein flour with high-protein flours.

	TURKEY					
No. of curve	1	2	3	4	5	6
Protein, %	8.3	9.3	10.2	11.2	12.1	13.2
	TENMARQ					
No. of curve	7	8	9	10	11	12
Protein, %	8.3	9.2	10.1	11.0	11.9	13.0
	CHIEFKAN					
No. of curve	13	14	15	16	17	18
Protein, %	8.3	9.2	10.1	11.0	11.9	13.1

Reducing High-Protein Flours to 11% by Blending with a 8.3% Protein Flour

The same low-protein soft wheat flour used in the preceding experiment was used to blend with several high-protein flours so that the mix-

tures were 11% protein. The protein and the absorption percentages are given in Table IV. The curves obtained on the original flours as well as the blends are given in Figure 5. In each pair of rows, the curves in the upper row are from the original flours and in the lower row from the 11% protein blend. The first two curves in the lowest row of Figure 5 are from the original flours and the last two from the 11% blend.

TABLE IV

EFFECT UPON ABSORPTIONS WHEN FLOURS WERE BLENDED WITH LOW-PROTEIN FLOUR TO A UNIFORM 11% PROTEIN CONTENT

Variety	Protein original	Absorption	
		Original	Blend
	%	%	%
Turkey	12.7	64.0	61
	13.2	63.0	60
	13.8	65.5	61
	15.6	69.5	62
Chiefkan	12.7	65.5	62
	13.1	68.0	63
	14.1	69.5	63
	15.4	72.5	63
Clarkan	12.2	57	57
	14.1	62	59
	15.4	64	60
Michigan Wonder	12.5	58	58
	14.8	65	60

The effects of diluting high-protein flours with a low-protein were similar to what was obtained by diluting with starch as shown in Figure 2. That is, all the curves from the blends are similar or very nearly so whether the dilution was from the higher- or the lower-protein flour. The figures for absorption were also nearly ıe same.

Discussion

The amount of water in a flour-water dough is about 40%–45% of the weight of the dough (Swanson, 1938). The greater part of this exists as adsorbed water on the starch and gluten, each probably holding equal amounts in a dough made from a flour of average or about 11% protein (Alsberg, 1927). It is assumed that in such a flour the protein holds as much adsorbed water as the starch, although this is present in six to seven times the amount of gluten (Swanson, 1938).

This assumption may explain why the absorptions are larger with an increase in protein. This adsorbed water is held on the surfaces of the starch and gluten material in varying degrees of freedom, depending on

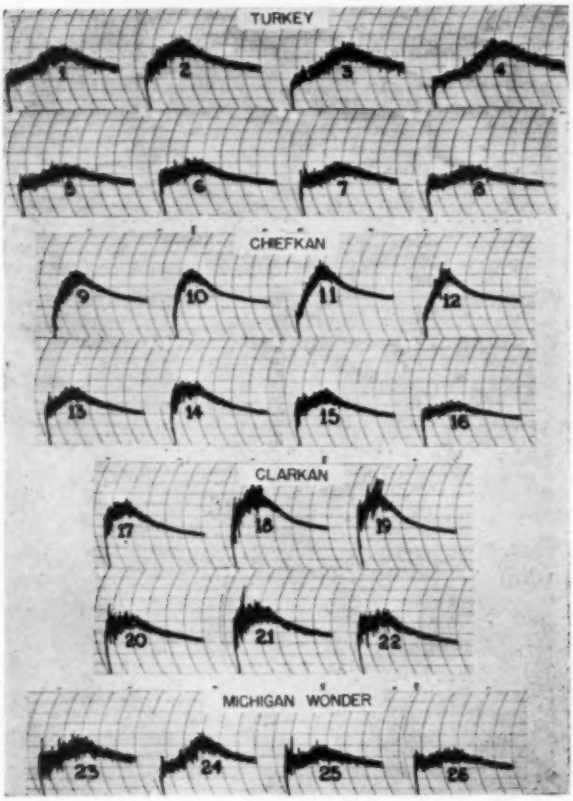

Fig. 5. Curves showing effect of reducing high-protein flours to 11% by blending with 8.3% flour.

TURKEY

	Original Protein				*Reduced to 11%*			
No. of curve	1	2	3	4	5	6	7	8
	12.7%	13.2%	13.8%	15.6%				

CHIEFKAN

	Original Protein				*Reduced to 11%*			
No. of curve	9	10	11	12	13	14	15	16
	12.7%	13.1%	14.1%	15.4%				

CLARKAN

	Original Protein			*Reduced to 11%*		
No. of curve	17	18	19	20	21	22
	12.2%	14.1%	15.4%			

MICHIGAN WONDER

	Original Protein		*Reduced to 11%*	
No. of curve	23	24	25	26
	12.5%	14.8%		

the distance from the adsorbing surface. The layers of water molecules next to the adsorbing surface are bound like the molecules in the starch and gluten material, since there is a possible chemical combination (Hauser, 1939). It is not known how deep this film of bound water is, but no doubt a considerable portion of the water in the dough is in this condition.

Such water does not function in giving the dough its characteristic properties. In the outer layers of water molecules away from the adsorbing surface, the freedom to move among others increases more and more until it is as great as in liquid water. That a portion of the water in the dough has this freedom is known from the fact that the vapor pressure of dough is equal to that of liquid water. It is the water which has this degree of freedom that contributes most to the characteristic properties of dough. The boundary line between free and bound water is not sharp (Hauser, 1939). Next to the surface the water molecules are held as though in chemical combination, but farther away from the surface there is a transition zone between the free and the bound water. In the inner part of this zone the molecules are more bound, and in the outer part they gradually approach the freedom that exists in liquid water. These partially bound water molecules in the outer layers may contribute something to the consistency of dough.

How much water is in the more free condition and how much is adsorbed or bound is not known, and as has been intimated the boundary line is not sharp. Calculations can be made to show that about one-third of the water in the dough is in the free condition (Swanson, 1938). Figures given by Alsberg (1927) indicate that gluten and starch each hold two-fifths of the total water and that one-fifth is free. This assumption, however, was based on work with soft wheat. Skovholt and Bailey (1935) used the term "bound water" in the sense that it is the difference between the total and free water present. They found the average bound-water value as a result of their determinations to be 51.4% of the total water present. This would indicate a much larger amount of water in the free condition than is often supposed. Vail and Bailey (1940) state that the average calculated bound water was found to be 35.5%. When water is added to make the dough, the bound-water demand would be satisfied first. This demand is probably fairly constant for any one flour. Any variation in absorption would have a multiple effect on the amount of free water, or the water that has most to do with the consistency of dough. It is for this reason that small deviations from optimum absorptions have an important effect on dough consistency.

The gluten in dough developed by mixing seems to exist as a three-dimensioned network (Swanson, 1925). When water first comes into contact with the protein to form gluten, this substance is arranged in a heterogeneous pattern. The mixing, when done in the pull-folding type of machine (the principle of most commercial mixers), orients these gluten strands into a more or less parallel system. The maximum resistance of dough to mechanical action apparently occurs when this parallel arrangement has been attained. The higher the protein and consequently the larger the quantity of the gluten strands, the greater the resistance, and hence, the higher the top of the curve. The dilution with starch or with a low-protein flour will attenuate this gluten network, causing a decrease in resistance, and hence the top of the curve will be lower.

Summary

The curves and data presented in this paper indicate the following:

The main characteristics of the curves obtained on the recording dough mixer with flours from sound wheats are determined by variety. That is, qualities inherent in a variety give curves of a certain pattern. Some varieties give patterns very similar to those of other varieties.

Within any variety the curves are influenced by the protein content and by absorption. A 2% variation in absorption will notably affect the height of the curve, but the main pattern due to varietal characteristics is not affected.

The protein content is the most potent factor within a variety. Given a reasonably correct absorption the height of the curve will be greater with increasing protein content. The distinctive varietal characteristics are more pronounced in the curves from medium- and high-protein flours than from low-protein flours. When high-protein flours are diluted with starch or a very low-protein flour so that the protein contents of the blends are equal, the patterns of the curves from the blends will be the same within a given variety, but the curves of the blends of one variety will be different from those of another variety, provided of course that the original flours from these varieties gave different curve patterns.

Theories are given that explain why variations in absorptions and protein contents influence the patterns of the curves.

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QUANTITY OF DOUGH IN RELATION TO THE USE OF THE FARINOGRAPH [1]

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(Read at the Annual Meeting, May 1940)

The farinograph has proved to be a very useful instrument in certain types of studies on wheat and flour quality and during the past ten years numerous papers have been published which have been based on farinograms. The characteristics of the curves which have been measured are primarily: (1) the height of the curve (oftentimes uniform at the maximum), (2) the time to reach maximum plasticity, (3) the slope of the curve from the peak to some arbitrary end as measured by areas or other means, (4) the width of the curve at various stages. Some authors have subjected the data thus obtained to statistical analyses in relation to baking-test data.

Judging from the details given in various publications containing farinograph data, the precaution of keeping the quantity of dough constant has apparently been overlooked except in the instance of Near and Sullivan (1935), who used a constant quantity of 480 g. of dough in their study of flour absorption. Others, and particularly Skovholt and Bailey (1932), have shown the necessity of maintaining the temperature constant for accurate results with the farinograph. This report will indicate that the quantity of dough is also a factor to consider. A comparison of results with the large 300-g. bowl and the small 50-g. bowl was also made, with identical conditions and materials.

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Experimental

Three flours were used with protein contents, at 15% moisture, of 14.83%, 10.87%, and 8.12%, respectively. Flour-water doughs with 2% salt were mixed at 30° and for 30 minutes. The ratios of each ingredient were calculated to give a total amount of 480 g. of dough when the large bowl was used and one-sixth of that quantity or 80 g. for the small bowl. When other amounts of dough were used the same ratios of the ingredients were also maintained for each flour.

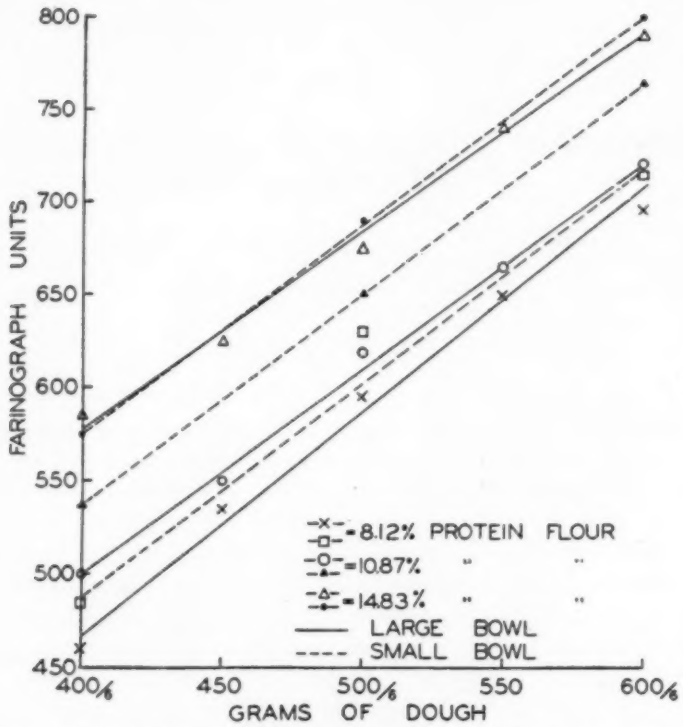

Fig. 1. The effect of the quantity of dough in the large and the small farinograph bowls on the height of the point of maximum plasticity in farinograph units. As the quantity of dough was varied the ratio of flour, total water, and salt was maintained constant for each flour.

Effect of the amount of dough.—Figure 1 shows the results of varying the amount of dough from 400 to 600 g. in the large bowl and from 66.6 g. (400/6) to 100 g. (600/6) in the small bowl. Farinograph units (F.U.) at the point of maximum plasticity are plotted against the quantity of dough. The slopes of the lines are approximately the same for all three flours and with both bowls.

Taking the average value of all the lines shown in Figure 1, the change in height of the farinograph curves per unit weight of the

doughs is 0.98 F.U. per gram of dough in the large bowl or per 0.167 g. in the small bowl. For all practical purposes it can be taken as one unit per gram or one unit per 0.167 g. in the large bowl and small bowl respectively.

Referring to the results obtained with the large bowl, the variation in the quantity of dough is usually not as large as from 400 to 600 g., but the variations generally are of significant proportions in ordinary work and can range from 450 to 550 g., thus causing a difference in height of the curve of up to 100 units. For instance, if a low-protein flour is mixed to give a flour-water dough with a maximum plasticity of 500 F.U., the absorption is about 50% on the fully corrected 15% moisture basis, thus giving a total quantity of dough of 450 g., which is perhaps the smallest quantity of dough generally encountered. The literature contains absorption figures of up to 70%, and sometimes higher, for high-protein flours. Such high absorption values are perhaps on the "as is" basis, but would give 510 g. of dough. Thus a difference of 60 g. is possible on flour-water doughs. When other ingredients are added such as salt, milk solids, sugar, malt, shortening, and others, the total quantity of dough can well be 550 g. or more. Since the height of the curve has been given so much significance, the necessity of controlling the quantity of dough becomes quite apparent for exact work and especially in research. The routine analyst might find it too cumbersome to adjust the ratios of ingredients to give a constant quantity of dough, although it appears desirable.

The quantity of dough used by Near and Sullivan (1935), 480 g. in the large bowl, appears to be optimum, since with larger quantities all of the dough is not agitated with each turn of the blades and this apparently accounts for the fluctuations in the 600 g. dough curves (or 600/6 dough curve) in Figure 2. These fluctuations were observed to a lesser extent in some 550-g. doughs.

The observations made with the large bowl also apply to the small bowl except that the quantity of dough considered is one-sixth of that used in the large bowl. In using the small bowl, it is apparent, of course, that any errors in weighing and measuring are six times as significant as when the large bowl is used.

Comparison of the large and the small bowls.—Figure 2 shows farinograms made from two flours with 400, 500, and 600 g. of dough in the large bowl and one-sixth of these quantities in the small bowl in the case of one flour. Each set of doughs was made to include exactly the same ratio of flour, water, and salt, and differed only as to the quantity of dough in the mixing bowl. The variation in height of the curve is quite evident.

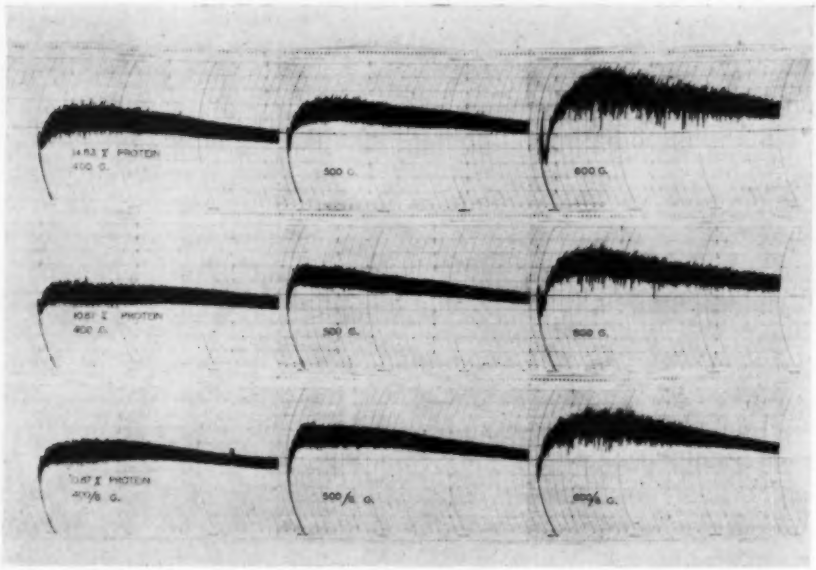

Fig. 2. Farinograms made with different quantities of dough prepared with the same ratio of flour, water, and salt. The two upper rows were made with the large bowl, and the lower with the small bowl. The two lower rows were made with the same kind and ratios of ingredients.

A comparison of the lower two rows of farinograms in Figure 2, made with the 10.87% protein flour, shows that curves obtained with the large bowl appear much like those obtained with the small bowl. Yet, a careful analysis of the curves made with the large and the small bowls reveals some variations in results. The measurements of curves made with two flours, and each flour at two absorption levels, are given in Table I. It is quite clear that the curves obatined with the small

TABLE I

COMPARISON OF THE LARGE AND THE SMALL FARINOGRAPH BOWLS

Flour protein content	Bowl	Absorption used	Time to maximum plasticity	Difference	Maximum plasticity	Difference	Plasticity at 30 min. mixing	Difference	Difference in plasticity at maximum and at 30 minutes
%		%	min.	min.	F.U.	F.U.	F.U.	F.U.	F.U.
14.83	Small	56	6.5	− 1	700	55	585	60	115
"	Large	"	5.5	—	645	—	525	—	120
"	Small	61	14	+ 2	510	30	445	0	65
"	Large	"	16	—	480	—	445	—	35
10.87	Small	50	2	+ 2	710	50	570	30	140
"	Large	"	4	—	660	—	540	—	120
"	Small	55	12.5	0	485	40	410	45	75
"	Large	"	12.5	—	445	—	365	—	80

bowl were not exactly identical with those obtained with the large bowl, although the ingredients were in the same ratios and the quantities of dough used were 480 and 80 g. for the large and the small bowls, respectively.

The farinograph records, as consistency units, the work required to turn the blades through the dough in the bowl. If more dough is used, even of the same consistency as maintained by a constant ratio of ingredients, more work will be recorded as consistency units. It is thus apparent that variations in weight of dough will cause corresponding variations in consistency units that have nothing to do with consistency, but rather with work input. In ordinary work these differences may not be important but when measurements are made of farinograms for statistical interpretations such differences might be significant.

The bowls used in this study were of the newer type with adjustments, and it has been pointed out to the authors that perhaps the adjustments were not properly made. However, the explicit directions made by the manufacturer were rigidly followed in using both bowls. Furthermore, if it were a question of adjustment, the differences between the small and the large bowl should probably be constant for various doughs, but this was not the case.

Mixing bowl cover.—In connection with this discussion it might be well to indicate the need of covering the mixing bowl while doughs are being mixed in it. In this laboratory a heavy piece of plate glass has been used as a cover and the heavy condensation of moisture on this during the 30-minute mixing period suggests an appreciable loss in moisture unless a cover is used. Such loss of moisture will affect the plasticity of the dough. This precaution of using a cover is of course quite imperative when the humidity of the laboratory atmosphere is low.

The need of using a constant quantity of dough for accurate work might also be true in case of other types of recording dough mixers.

Summary

Plasticity of the dough as recorded by the farinograph is an almost linear function of its weight when the ratio of ingredients is kept constant. It is found to increase very nearly one farinograph unit per gram of increase in the weight of dough.

Constant quantity of dough is shown to be essential in the accurate use of the farinograph, just as it has previously been shown that constant temperature is essential to accuracy.

Optimum charge of ingredients in the large bowl appears to be 480 g. and one-sixth of that quantity, or 80 g., in the small bowl.

Curves made on the same flour with a constant ratio of ingredients at three weight levels with both bowls were not identical when comparable precise measurements were made of them.

Covers for the bowls are recommended to prevent loss of moisture from the doughs during a test.

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SOME STUDIES ON FLOUR ABSORPTION [1]

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(Read at the Annual Meeting, May 1940)

Many attempts have been made to standardize a technique for the determination of the value generally referred to as flour absorption, and Near and Sullivan (1935) and Bailey (1940) discuss the various instruments and methods that have been used. The absorption of a flour can be considered as the amount of liquid, accurately calculated and expressed on a standard flour moisture basis, that is required to give a dough with proper handling and machining properties and that will produce the best final baked product. The value varies with formula and baking method as well as with the procedure used. Thus in view of the many types of bakery products and baking procedures, it naturally becomes difficult to express the absorption of a flour as a single value. Yet a single relative value of the water-absorbing capacity of a flour, based on a standard formula and technique, becomes quite useful even when other formulas and methods are used, and such procedures have been generally adopted.

This is a study of doughs from several flours made at different absorption levels as compared by the use of the farinograph, the pressure plastometer described by Stamberg and Bailey (1940), and by various baking tests.

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Calculation of Absorption

Before presenting the experimental data it appears pertinent to discuss the methods of calculating absorption. It seems that three different methods have been used in establishing absorption values, although only one can be considered correct, and unfortunately in many publications the method used has not been stated. The three methods and an example of calculating each are listed:

1. The fully corrected method, basis 15% moisture. Let the flour contain 12% moisture. Then 96.6 g. contains 85 g. of dry matter. One must add 3.4 g. of water to make the total equal 100 g. Whatever water over and above this amount that is used in making the dough is all that can be called the absorption of 100 g. of flour, basis 15% moisture. If 58 g. of water is used, beyond the 3.4 g. already specified for correction of flour weight, then 58 is the percentage of absorption. For any moisture content of the flour the absorption value remains a constant.

2. The dry matter corrected method, basis 15% moisture. Let the flour contain 12% moisture. Then, as before, 96.6 g. of flour contains 85 g. of dry matter. To make up a dough, as before, $3.4 + 58 = 61.4$ g. of water is used, and 61.4 is given as the percentage of absorption.

3. The "as is" basis. Again, let the flour contain 12% moisture. If a dough of the same plasticity were made up, using 100 g. of flour, the quantity of water required would be $(61.4 \div 96.6) \times 100 = 63.7$ g. This is reported as the percentage absorption on the "as is" basis. It is readily seen that as the flour loses moisture, its "as is" absorption increases, leading to a fictitious value for the flour.

TABLE I

FLOUR ABSORPTION BY DIFFERENT METHODS OF CALCULATION

Flour	Moisture content of flour	Absorption, 15% moisture basis, fully corrected	Absorption, 15% moisture basis, dry matter corrected	Absorption on "as is" basis
	%	%	%	%
1	8.4	56.0	63.3	68.3
2	10.1	56.0	61.5	65.5
3	11.0	56.0	60.5	63.4
4	12.0	56.0	59.4	61.5
5	14.0	56.0	57.1	57.8

The difference in values obtained by these three methods depends of course on the moisture content of the flour as can be seen by the calculated hypothetical values in Table I. When the moisture content of the flour is low, or 8.4%, the absorption values range from 56.0% to 68.3%, or a difference of 12.3%. When the moisture content of the flour

approaches the moisture basis used the difference is smaller, as at 14% moisture the difference is only 1.8% in the absorption values.

The apparent use of all three methods perhaps accounts for the rather wide range of absorption values occurring in the literature, and it appears quite desirable to use the fully corrected method since such data could then be used to find the correct ratio of liquid to dry matter.

In this laboratory a graph is used to find the correct weight of flour to use in order to provide 85 g. of dry material at various moisture levels from 0% to 15%. It is not a straight line relationship as often

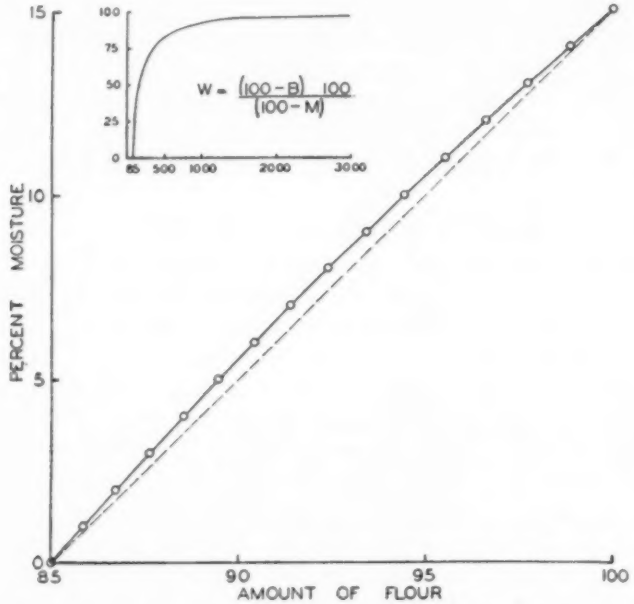

Fig. 1. The continuous line of the large graph passes through plotted values of flour weights containing 85 g. of dry matter for percentage moisture content, ranging from 0% to 15%. The broken line indicates the departure of the graph from that of a straight line. The small graph plotted in the same way, with ordinates as moisture content of flour, abscissas as grams of flour, but over a range of moisture content of flour from 0% to near 100%, shows the hyperbolic nature of the curve.

believed, but slightly curvilinear as shown in Figure 1, and it is part of a hyperbola of the general equation:

$$W = \frac{(100 - B)\ 100}{100 - M},$$

where W = the weight of flour to use to provide a constant weight of dry material.

B = flour moisture basis used. The large graph in Figure 1 is plotted for the 15% moisture basis with a range in moisture content of flour from 0% to 15%.

M = percentage moisture in the flour.

The same formula can be used, of course, to obtain the curve for any other moisture basis. All absorption values included in the subsequent paragraphs are on the 15% moisture basis and by the fully corrected method.

Farinograph and Plastometer Tests

A series of twelve commercially milled flours ranging in protein content from 7.26% to 15.50% at the 15% moisture basis were used. Flour-water doughs containing 2% salt were mixed in the farinograph at 30° C. and the percentage absorption necessary to give a maximum plasticity of 500 F.U. was determined. The ratio of ingredients was adjusted to provide 480 g. of dough in accordance with the recommenda-

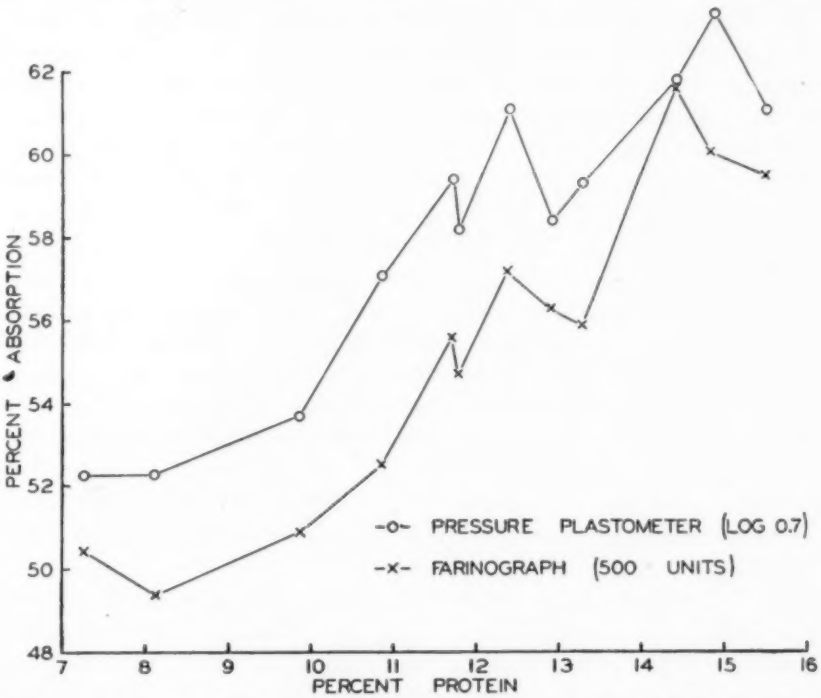

Fig. 2. Relationship of absorption to protein content of various flours as based on measurements on the farinograph and the pressure plastometer.

tions of Stamberg and Merritt (1941). The doughs used in the pressure plastometer were first mixed in the Hobart-Swanson mixer for two minutes and then used at 30° C. The absorptions equivalent to a rate of flow of 5.01 g. per minute (log. 0.7) were determined as described by Stamberg and Bailey (1940).

The graph in Figure 2 shows the relationship between the percentage absorption and the protein content of the flours from the resulting data.

Employing the criteria of a constant rate of flow and a constant plasticity in the farinograph there is obviously an increase in absorption with higher percentages of protein. On the average, this change amounts to about 1.5% absorption per 1% of flour protein.

The results with the farinograph and the plastometer are very much the same and show the same general trend. By selecting other reference standards than 500 F.U., or rate of flow per minute, the curves could be made almost to coincide.

Baking Tests

Baking tests were carried out at various moisture levels for each flour. The formula used included 1% salt, 5% sugar, and 3% yeast. The doughs were mixed for 2 minutes in the Hobart-Swanson mixer. A survey bake was made with the 12 flours using 1½, 2, and 2½ hours' fermentation to find the optimum time, with the results that the three flours of lowest protein content were given 1½ hours of fermentation and all others 2½ hours. The proofing period was 55 minutes at 30°, followed by baking for 25 minutes at 230° C. A mechanical sheeter was used for punching and molding. The low-form baking pans described by Markley (1940) were used.

Each flour was baked at various absorption levels, in one case using a 150 g. portion of dough and in the other case using all of the dough obtained from a unit weight of flour dry matter with the consequent variation in water. The loaves were scored as to volume, texture, grain, and loaf type and expressed in single quality scores, but these values were practically in direct relationship to the loaf volumes which have been used in the data plotted in Figure 3.

It is interesting to note from Figure 3 that when a constant weight of dough was used the larger loaf volumes were reached with increased percentages of absorption, followed by decreased loaf volumes with higher absorption, except in the case of the two soft-wheat flours with 7.26% and 8.12% protein, in which cases the handling property of the dough was the limiting factor. However, when all of the dough was used such maximum loaf volumes followed by decreased values are not apparent. The highest absorptions used were about 64% and 65% on the 15% moisture basis stated in terms of the fully corrected method. The absorption would be much higher if stated on the less accurate dry-matter-corrected or "as is" methods, or on the basis of 13.5% moisture. Thus sufficiently high absorption levels were studied.

The baking data show that when a constant quantity of dough was used the optimum absorptions for the flours were at about 57%–58% on the 15% moisture basis and with the formula and method used.

This indicates that on the accurately calculated and fully corrected absorption value at the 15% moisture level, the optimum absorption is about the same for widely different flours.

Taking the optimum absorption values from the baking data for which a constant quantity of dough was used, a series of farinograph tests was made. These absorption values were all close to 58%. In

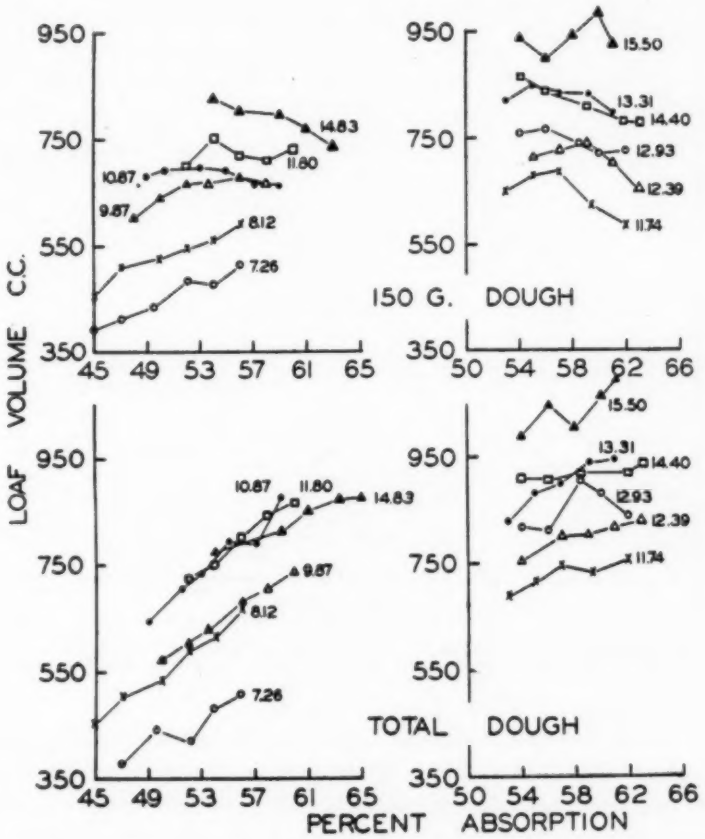

Fig. 3. Loaf volumes at various absorption levels of 12 flours with different protein contents, the upper graphs with a constant amount of dough, and the lower graphs with all of the dough from 85 g. of flour, dry basis.

Figure 4 the resulting maximum plasticity of the farinograph curve is plotted against the protein content of the flour. It is evident that when optimum absorptions are used on the basis of the baking results the plasticity of the dough increases with higher protein contents of the flours. This is substantially in agreement with the results of Merritt and Bailey (1939), showing that weak flours produce the best baking

results at absorptions giving a low plasticity in the farinograph and stronger flours at an absorption giving correspondingly higher plasticities. Many practical bakers also mix low-protein flours to give slacker doughs, and high-protein flours to give stiffer doughs for best results.

The data in Figure 3 indicate the interesting possibility that for test baking a fully corrected absorption of 58% on the 15% moisture basis

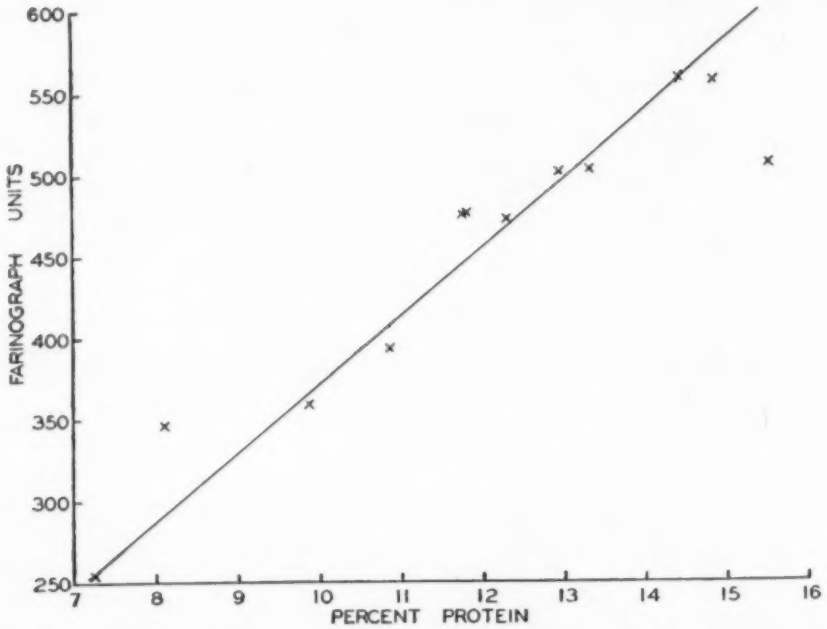

Fig. 4. Relationship between protein content of flour and maximum plasticity, in farinograph units, of doughs mixed at the absorption level found to be optimum by the baking tests for the different flours.

could be used for all flours, and particularly when a constant quantity of dough is used, thus eliminating variations in absorption. It is claimed by many that a better spread in values or differentiation of flour quality is obtained when all of the dough is used in test baking. Such possibilities as are suggested in this paragraph must, of course, be examined with numerous samples of flour.

Summary

Calculation of absorption values of flours by three methods are in general use but only one is entirely correct.

Absorptions of twelve flours, ranging from 7.26% to 15.50% protein content, necessary to have a constant plasticity in the farinograph and

a constant flow in the pressure plastometer, were found to increase about 1.5% for each 1% increase of protein content.

Baking tests were carried out using the flours at various absorption levels, and at each level loaves were baked first with a constant amount of dough of 150 g. and, secondly, from all of the dough resulting from 85 g. of flour, dry basis. With the first method, peaks of optimum absorption were observed as judged from the bread quality, but with the second method no peaks were noticed over the absorption range studied.

Optimum absorptions resulting from the baking tests with a constant amount of dough were found to give doughs of low plasticity in the farinograph for low-protein flours and increasingly higher plasticities for higher-protein flours.

The possibility of using a constant absorption and a constant amount of dough in test baking for flour quality is suggested.

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THE EFFECT OF PROTEIN CONTENT ON THE BAKING BEHAVIOR OF SOME WINTER WHEAT VARIETIES [1]

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Since protein content is the most significant single factor influencing baking performance of sound flours, a consideration of protein content in the correct evaluation of wheat samples is important. Larmour, Working, and Ofelt (1939, 1940) reported upon a series of Kansas-grown varieties of the 1938 crop. Their studies (1939) demonstrated " that within a given season the potential strength of the principal hard red winter varieties is related to protein content in a linear fashion, and is very highly correlated with it." In a second paper (1940) dealing with soft winter wheat varieties these workers stated that a linear relation between loaf volume and protein content did not exist because the loaf volume tended to recede above approximately 10% flour protein in the soft wheat varieties studied.

The study reported at this time was undertaken with two objectives in mind: (*a*) to see if the conclusions reported by Larmour, Working, and Ofelt (1939, 1940) would hold for the 1939 Kansas crop, and (*b*), to observe the effect of the use of a uniform mixing time upon the behavior of a series of flours of varying protein amounts and qualities. An excellent series of wheat samples was made available for this study through the co-operation of A. L. Clapp of the Department of Agronomy.

A review of the literature at this time appears unnecessary because of the comprehensive review given by Larmour (1940). He concluded that the lack of linearity formerly thought to exist between protein content and loaf volume was due to inadequate baking methods. Recently, McCalla (1940), in a study of hard red spring wheats, concluded that the protein content-loaf volume regression coefficient for a given variety is as much a varietal characteristic as is yield or protein content. High-protein Reward wheat (15% and above) made bread of large volume, equal to or better than Marquis, but low-protein Reward was not much, if any, better than Garnet. Garnet required more protein at all levels than Marquis or Red Bobs in order to make a loaf of specified volume.

Anderson, Sallans, and Ayre (1938) used linear regressions with scatter diagrams effectively to show the relationship of nitrogen and sac-

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charifying activities in a study of varietal differences in barleys and malts. A similar arrangement proved useful in showing results graphically in the present study (Fig. 3).

Materials and Methods

Samples of the principal varieties of wheat grown in Kansas were produced by farmers in large plots from pure seed supplied by Kansas State College at 59 locations in Kansas. Hard red winter varieties included were Turkey, Tenmarq, Cheyenne, Kanred, Nebred, Early Blackhull, Blackhull, Chiefkan, and Iobred, whereas Kawvale, Michigan Wonder, and Clarkan represented the soft red winter wheats.

Small samples of each variety were harvested and shipped to the Experiment Station for threshing so that acre yields, protein content and other determinations could be made. The samples according to variety were then composited according to their protein contents at about the following protein levels:

Below 10%	14.6 to 16.0%
10.1 to 11.5%	16.1 to 17.5%
11.6 to 13.0%	17.6% and above
13.1 to 14.5%	

These composites were large enough to provide a milling sample in most cases. Five or more flours of each variety were available for baking excepting Nebred and Iobred. It was recognized that this method of making the composites produced samples which were not strictly comparable, as the same locations were not necessarily represented in the various protein levels of the different varieties. Differences in growing environments may account for some of the inexplicable baking results obtained. Samples weighing less than 53 pounds per bushel were discarded as well as all musty or excessively shrivelled samples. The samples were thoroughly mixed and then milled on a Buhler mill. The straight-grade flours were analyzed, stored in tightly covered containers at room temperatures for two weeks and then kept in a cold room at approximately 41°F. until baked.

The flour samples were baked by two methods differing only in the length of mixing time. In one method the doughs were mixed for three minutes at 80 rpm. regardless of the handling properties or stage of development of the doughs. The three-minute period (240 revolutions of mixer) selected as the fixed time was based on a preliminary study involving varieties of widely different mixing times. In the other method the doughs were mixed at 80 rpm. to the optimum consistency as determined by observation. A Swanson-Working dough mixer with a bowl containing two adjacent pins was used.

The baking formula used in both methods was as follows:

Flour	100 g.
Yeast	3 g.
Salt	1 g.
Sugar	6 g.
Malt syrup (120° L.)	0.25 g.
Dry milk solids	6 g.
Shortening (hydrogenated vegetable)	3 g.
Potassium bromate	0.004 g.
Water (distilled)	As needed

Absorption values as determined for the optimum mixing method were also used in the fixed-mixing-time bakes. The standard A.A.C.C. baking test fermentation times and temperatures were employed. Loaves were baked in an electric oven for 24 minutes at 430°F. Fermentation bowls, fermentation cabinet, tall-form baking pans, and the loaf volume measuring apparatus used complied with the requirements of the A.A.C.C. method. A National pup sheeting roll was used for punching, and molding was done on a Thompson Model A laboratory molder. Loaf volumes were measured immediately after the loaves were removed from the oven. The final loaf volumes reported are averages of at least two loaves from two bakes made on different days. Additional replicated bakes were made in cases where the volumes differed more than 20 cc. from each other. The loaves were scored the following day for external and internal characteristics.

Baking absorption values were determined by adding proportionate amounts of the same ingredients used in the baking formula to 20 g. of flour in the bowl of a National micro mixer. Distilled water was added and the mixer operated until the proper consistency as determined by "sight and feel" of dough was attained. The resulting doughs were then fermented for three hours and the final desired baking absorption then estimated from the consistency of the dough. This method is similar to that devised and used by Karl F. Finney[3] of the Federal Hard Winter Wheat Quality Laboratory.

Dough-mixer curves were made for all flours, using a National-Swanson-Working recording micro mixer. All curves were made at the No. 9 setting. Thirty-five g. of flour was used and distilled water was added to equal the absorptions used in baking.

The time-test determinations were made according to the procedure described by Swanson (1937). Test weight, protein, moisture, and ash determinations were made by approved methods. The percentages of protein and of ash are reported on a 15% moisture basis.

[3] The authors express their appreciation to Karl F. Finney, Agent, U. S. Department of Agriculture, for details of his absorption method.

Discussion of Results

Analytical data for wheat and flour are given in Table I. These data show low correlation between protein content and the results obtained by the time test. There was, however, a tendency for longer time to be associated with higher protein content. Percentages of flour yield and flour ash varied somewhat, being influenced undoubtedly by milling and by test weight. Absorption values, while somewhat irregular, showed a definite trend to increase as the amount of protein increased. Typical soft wheats such as Michigan Wonder or Clarkan had lower absorptions than good hard varieties. Kawvale absorbed more water

TABLE I

ANALYTICAL DATA

Serial No.	Wheat					Flour			
	Test weight	Flour yield [1]	Time test	Moisture	Protein [1]	Moisture	Protein [1]	Ash [1]	Baking absorption
	lbs.	%	*min.*	%	%	%	%	%	%
				TURKEY					
25095	—	69.4	74	10.6	9.7	14.0	8.8	0.453	56.2
96	58.4	70.8	72	10.8	10.6	13.8	9.5	0.485	55.8
97	57.8	69.3	67	10.6	11.9	13.7	10.5	0.457	57.6
98	57.5	69.7	101	10.6	13.8	13.6	12.7	0.461	59.4
99	57.5	68.5	99	11.0	14.7	14.1	13.9	0.497	63.3
100	56.8	67.9	115	10.2	16.6	13.4	15.7	0.503	63.9
101	57.1	68.3	115	10.4	17.5	13.5	16.7	0.490	65.1
				TENMARO					
89	58.2	66.1	135	10.6	10.8	14.6	9.8	0.416	60.2
88	—	70.2	123	10.5	11.1	14.2	10.7	0.440	59.5
90	58.5	66.9	110	10.6	11.9	13.8	11.2	0.432	58.8
91	59.6	69.2	100	10.8	13.4	13.7	12.3	0.413	59.6
92	57.6	67.9	125	10.4	14.9	13.4	13.7	0.404	61.0
93	57.1	66.5	145	10.2	15.7	13.5	15.3	0.437	63.1
94	56.1	64.4	145	10.4	17.6	14.0	16.6	0.474	67.0
				KANRED					
63	57.6	71.1	72	10.4	10.9	14.2	10.2	0.450	60.5
64	57.8	72.2	72	10.6	11.9	13.9	10.8	0.409	59.9
65	57.8	67.9	71	10.5	13.6	14.3	12.5	0.410	62.7
66	57.4	70.2	57	10.5	14.9	13.8	13.9	0.434	63.7
67	59.4	69.2	90	10.8	16.3	14.2	15.5	0.444	66.4
68	58.2	67.7	73	10.2	18.0	13.9	16.4	0.474	66.8

[1] Moisture basis 15%.

TABLE I—*Continued*

Serial No.	Wheat					Flour			
	Test weight	Flour yield [1]	Time test	Moisture	Protein [1]	Moisture	Protein [1]	Ash [1]	Baking absorption
	lbs.	%	*min.*	%	%	%	%	%	%
				CHEYENNE					
25049	58.8	69.0	122	10.6	10.6	13.9	10.1	0.430	56.0
50	57.8	72.1	98	11.3	11.9	13.6	10.9	0.417	56.4
51	59.5	69.6	125	10.5	13.3	13.7	12.1	0.441	57.6
52	59.2	68.8	104	10.6	14.9	14.0	13.9	0.453	59.1
53	59.6	67.3	114	10.5	16.3	13.8	15.3	0.465	60.7
54	58.6	70.9	132	10.5	17.3	13.5	16.2	0.505	62.1
				NEBRED					
84	—	68.7	121	10.4	13.5	14.1	12.7	0.519	62.3
85	60.1	69.5	125	10.4	14.8	13.8	13.9	0.450	60.7
86	58.6	68.6	154	10.3	16.7	13.4	15.8	0.507	62.0
87	57.6	70.4	176	10.4	17.7	12.9	16.9	0.531	62.0
				EARLY BLACKHULL					
35	—	66.5	77	10.3	10.9	13.9	10.4	0.438	56.0
36	58.6	66.4	56	10.6	12.0	14.6	11.5	0.394	58.3
37	60.8	62.7	56	10.7	13.4	14.3	12.6	0.387	58.7
38	60.0	64.0	66	9.9	15.0	14.0	14.6	0.411	59.1
39	58.1	65.0	91	11.1	16.2	13.7	15.6	0.435	59.6
				BLACKHULL					
29	59.4	65.7	63	10.4	11.9	14.8	11.2	0.401	58.6
30	59.0	64.7	78	10.3	13.8	13.7	12.3	0.414	56.6
31	58.6	62.0	54	10.3	14.9	14.4	14.4	0.427	61.9
32	59.0	62.8	74	10.4	16.4	14.4	15.8	0.441	61.9
33	58.1	62.2	111	10.2	17.2	14.4	16.7	0.457	63.8
				CHIEFKAN					
42	61.0	70.7	60	10.7	11.1	14.0	10.7	0.423	61.1
43	60.6	71.0	58	10.6	11.8	13.8	11.1	0.434	61.7
44	59.4	71.8	61	10.1	13.2	14.0	12.8	0.405	63.1
45	59.6	70.1	61	10.5	15.0	14.6	14.2	0.436	64.2
46	59.3	69.0	70	10.3	16.1	13.6	15.6	0.545	63.3
47	59.9	66.6	65	10.3	16.9	14.2	16.1	0.466	62.5

TABLE I—*Continued*

Serial No.	Wheat					Flour			
	Test weight	Flour yield [1]	Time test	Moisture	Protein [1]	Moisture	Protein [1]	Ash [1]	Baking absorption
	lbs.	%	*min.*	%	%	%	%	%	%
				IOBRED					
25076	60.0	65.0	78	10.6	13.8	13.9	12.7	0.355	58.9
77	—	62.8	—	10.5	15.2	13.5	14.0	0.350	60.2
				KAWVALE					
70	58.3	69.0	71	10.5	10.5	13.8	10.2	0.462	58.8
71	56.0	68.2	75	10.7	12.2	13.6	11.2	0.449	61.3
72	56.6	66.6	83	10.7	13.6	13.8	12.3	0.418	62.7
73	56.0	65.8	128	10.7	15.2	13.3	14.0	0.443	62.7
74	55.0	68.1	104	10.6	15.4	13.5	14.5	0.470	65.1
				MICHIGAN WONDER					
79	57.0	61.8	42	10.4	10.5	13.3	10.1	0.396	55.9
80	57.6	61.4	54	10.3	11.9	13.2	10.8	0.401	56.7
81	58.2	63.3	59	10.3	13.4	13.0	12.3	0.399	57.3
82	56.8	68.3	70	10.4	14.7	13.6	13.5	0.437	60.4
83	56.8	60.7	76	10.2	15.8	13.0	15.0	0.422	59.3
				CLARKAN					
56	59.6	54.2	49	10.4	10.9	14.1	10.0	0.350	57.3
57	60.3	56.7	53	10.2	12.2	13.7	10.4	0.371	56.6
58	59.1	60.5	51	10.3	13.4	13.7	11.9	0.382	56.6
59	59.1	61.3	71	10.4	15.1	13.4	13.6	0.379	57.0
60	58.1	61.5	74	10.3	16.5	13.2	15.4	0.407	57.7

[1] Moisture basis 15%.

than the typical soft wheats. It is evident that varieties cause definite differences in absorption values.

Figures 1 and 2 show the loaf volumes for the various varieties at different protein levels, excepting Nebred and Iobred, for which less than five levels of protein were available. Neither of these two varieties proved outstanding and their inclusion would have merely confused the figures. Loaf volumes for Nebred with the fixed mixing time were, with increasing protein content, 855, 868, 1,072, and 1,112 cc.; with optimum mixing time, 815, 870, 1,033, and 1,145 cc. respectively. Similarly, with Iobred the results were 898 and 1,013 cc. for the fixed mixing time, and 895 and 975 cc. for the optimum mixes.

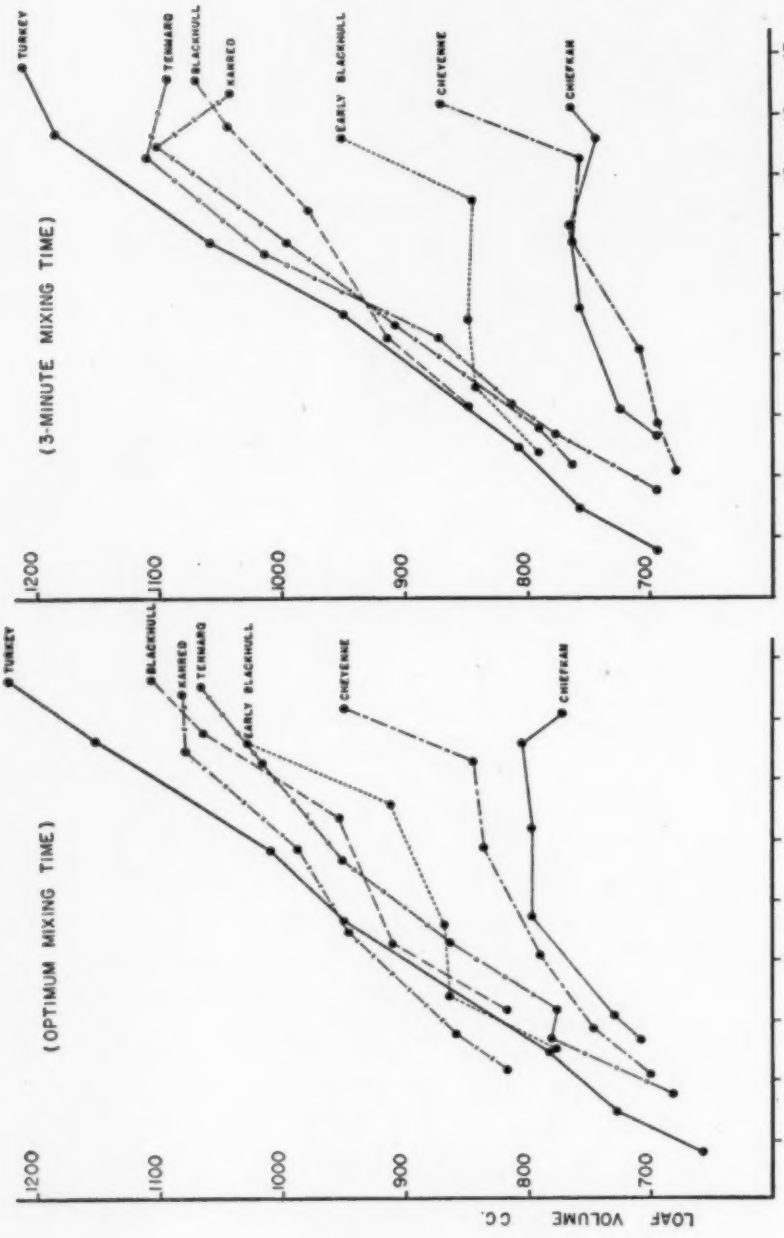

Fig. 1. Loaf volumes for hard red winter wheats at various protein levels (1939).

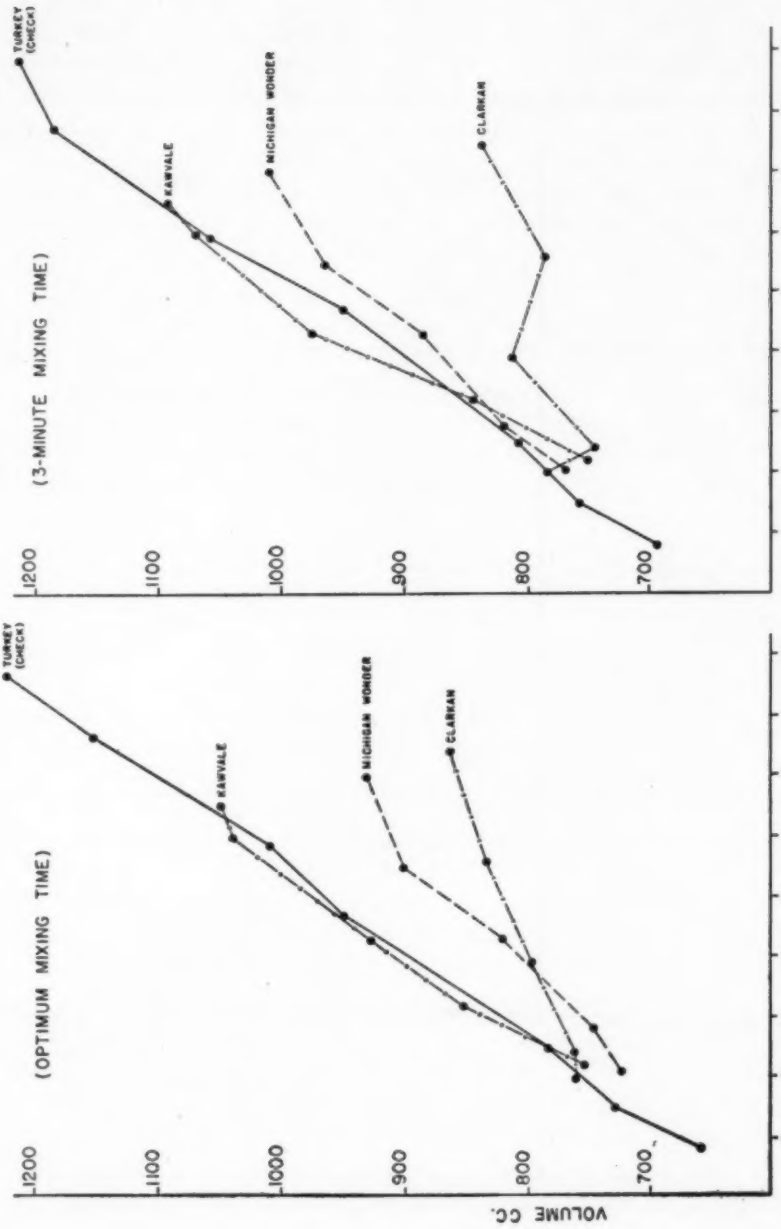

Fig. 2. Loaf volumes for soft winter wheats at various protein levels (1939).

With the fixed three-minute mixing method linearity between loaf volume and protein content is evident in most of the varieties studied (Fig. 3). Turkey, Tenmarq, Cheyenne, Kanred, Blackhull, Kawvale, and Michigan Wonder may be noted in this respect. With Clarkan and Chiefkan, increasing protein content had the least effect upon the loaf

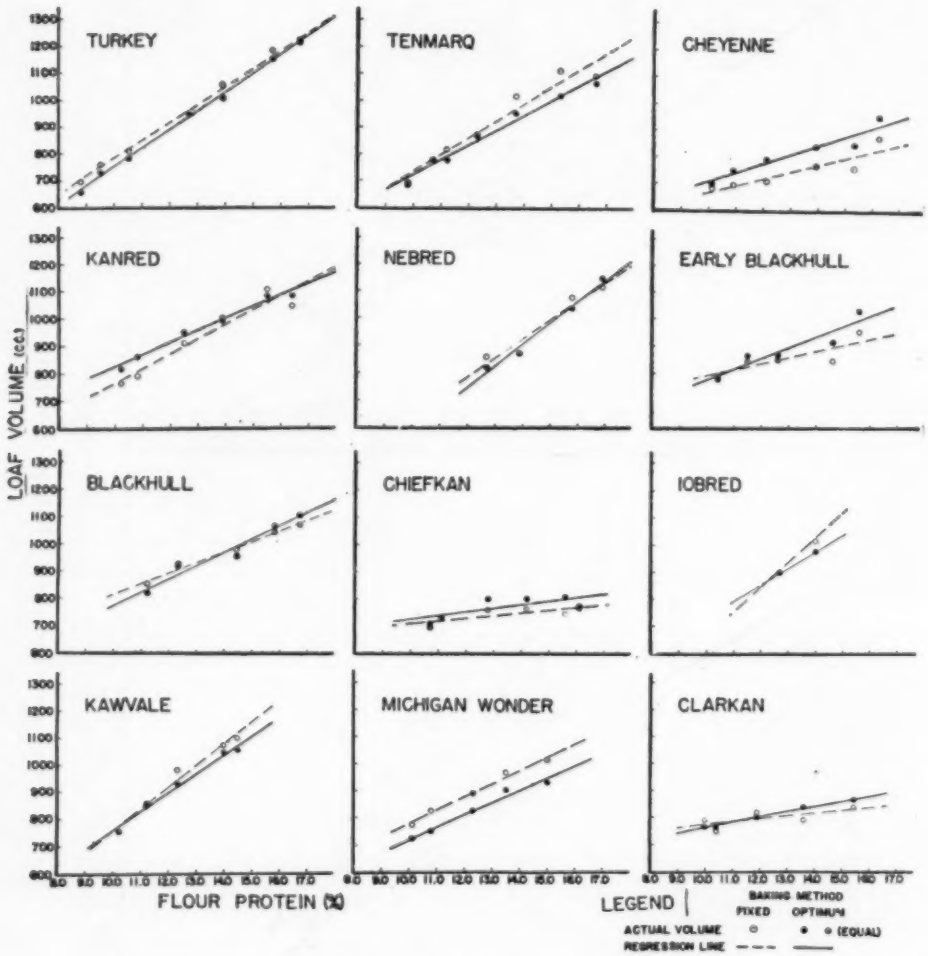

Fig. 3. Protein-loaf volume regression lines from two baking procedures.

volume. No reason is evident for the peculiar behavior exhibited by Early Blackhull. The figures indicate linearity between loaf volume and protein content in the same varieties when baked with the optimum mixing time as with the fixed mixing time. The loaf volumes of Clarkan and Chiefkan were little affected by the protein content with either mix-

ing time. Generally speaking, it would appear that the use of the optimum mix tends to improve somewhat the behavior of such varieties as Chiefkan, Early Blackhull, and Cheyenne, particularly at the higher levels of protein.

Cheyenne was the only variety noticeably undermixed with the fixed mixing time (240 revolutions of mixer). The doughs were dead and underdeveloped. By mixing to the optimum the handling properties of the doughs of Cheyenne and the resulting bread were improved. Chiefkan, Blackhull, Early Blackhull, Michigan Wonder, and Clarkan with three minutes of mixing time generally produced doughs that were overmixed, sticky, and difficult to handle. However, these varieties, even when overmixed, produced bread similar in quality to that from doughs mixed to the optimum. The optimum mixing time required varied between 1.75 and 5.0 minutes for the entire series of samples and generally the mixing time, within a variety, tended to decrease as the protein content increased. Examination of the curves (Figs. 4 and 5) shows this same tendency for decreasing mixing requirements with increasing protein content.

A linear relationship between protein content and loaf volume for most varieties included in this study has already been indicated. However, it is also evident from Figures 1 and 2 that the ranking of the varieties is somewhat different for each baking method at each protein level. In this respect the baking methods cannot absolutely be used interchangeably in experimental baking for rating varieties as to baking quality. It is interesting to note that some of these flours when baked by Sandstedt and Ofelt (1940) at Lincoln, Nebraska, with a formula similar (except for type of malt used) to that used in these studies, gave results and rankings different from these same flours when baked at Manhattan, Kansas. Sandstedt and Ofelt used malted wheat flour as the diastatic supplement. Methods of handling the doughs also differed somewhat. It becomes increasingly evident that great care is needed in the choice of test procedures to evaluate varieties so that these varieties will be well adapted to their uses by the consuming public.

Type of Regression Lines

McCalla (1940) has pointed out that the relation between protein content of wheat and loaf volume of bread is just as much a variety characteristic as are yield per acre and protein content. Data presented in this paper seem to substantiate the above-mentioned relationship in regard to hard red winter varieties. Regression lines were calculated by a method given by Fisher (1936) to investigate this point. Figure 3 shows the actual loaf volumes as well as regression lines for each mixing

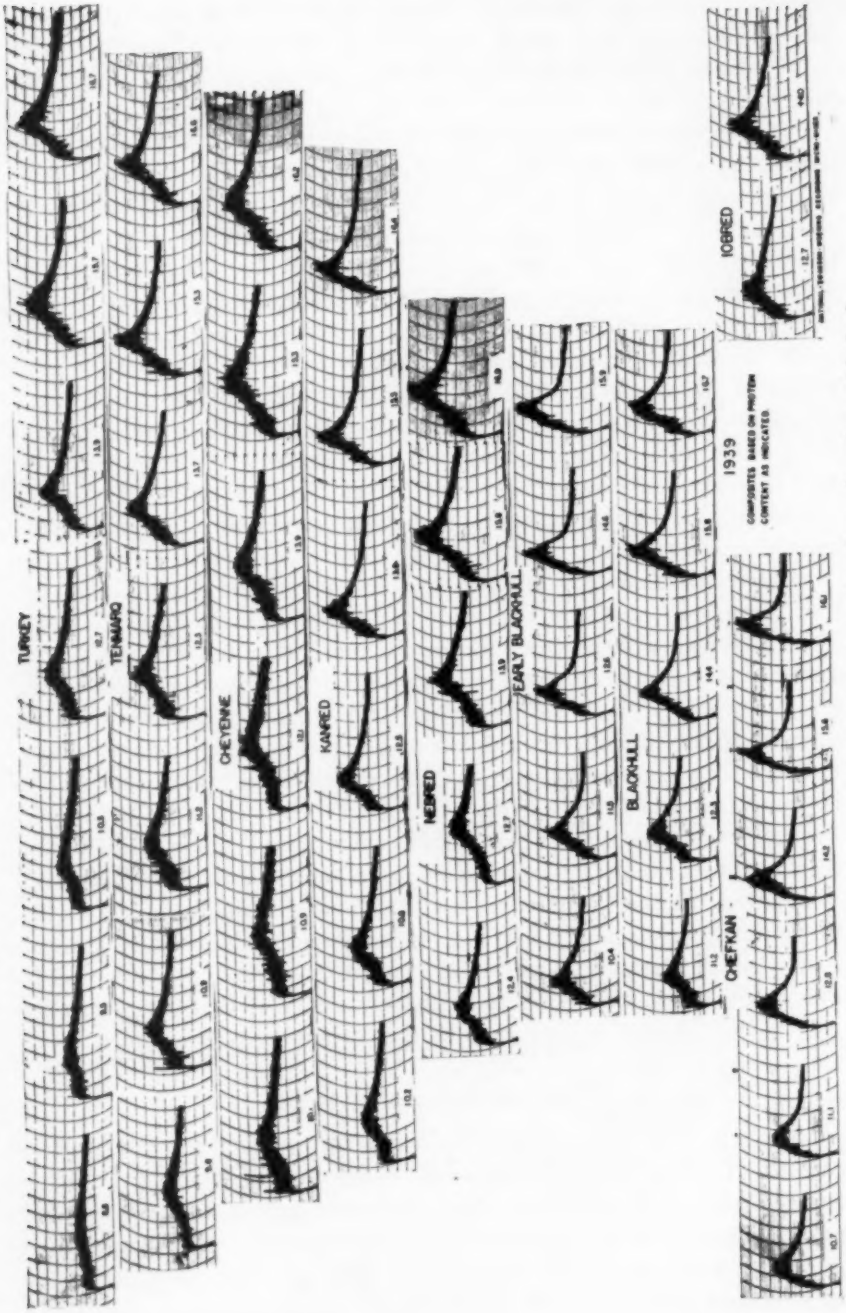

Fig. 4. Micro-mixer curves of hard red winter wheat flours of varying protein levels.

method. The actual data fit the calculated regression lines very well in most instances.

These regression lines show distinctly that the loaf volumes of the different varieties are not affected to the same extent by increases in protein content. These regression lines may be grouped into three general types: (1) lines which demonstrate that loaf volume is relatively poor at lower protein levels and is greatly improved at the higher protein levels, *e.g.* Nebred; (2) lines which demonstrate that loaf volume is relatively good at low protein levels and improves little with increasing protein content, *e.g.* Chiefkan and Clarkan; and (3) lines which demonstrate a uniform and considerable increase in loaf volume in relation to

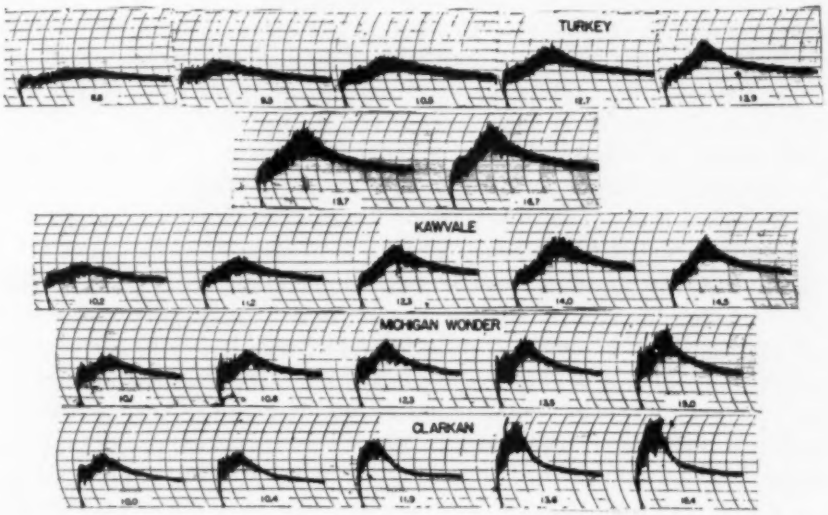

Fig. 5. Micro-mixer curves of Turkey, Kawvale (a semihard variety) and two soft winter wheats, Michigan Wonder and Clarkan.

protein content throughout the entire protein series, *e.g.* Turkey. The baking performances of varieties with regression lines similar to Turkey may be more readily forecast from a protein determination than the performance of varieties possessing either of the other types of regression lines. Further work with hard red winter wheats will be needed to determine definitely that type of regression line is an inherited characteristic in this class of wheat. It is evident that the baking method will influence the type of line to a material extent.

Micro Mixer Curves

Mixing curves for these flours are shown in Figures 4 and 5. These were obtained on the Swanson-Working recording micro mixer made

by the National Manufacturing Company. This machine differs from the original recording micro mixer used by Larmour, Working, and Ofelt (1939) chiefly in that the chart paper is drawn through three times as fast, making each division equal to about one minute of mixing time. The mixing head also rotates at a slightly higher speed. The combined effect of these two changes is that the curves are spread out to approximately twice the length as compared with the curves made with the original model of the recording micro mixer.

No entirely satisfactory method has so far been developed for describing micro-mixer curves. A reliable interpretive method whereby the curves may be expressed numerically is needed. The curves give a pictorial expression of some dough-handling properties of a flour and in this respect the micro mixer provides information about a flour which hitherto has been lacking. Dough-handling properties are factors of great importance to the baker using commercial machinery. They are of less importance in the laboratory testing of samples. Geddes, Aitken, and Fisher (1940) and Geddes (1940) have pointed out that dough-handling properties are not necessarily correlated with the characteristics of the baked loaf. That this is true is exemplified by the curve type and baking behavior of Cheyenne.

Curves indicate approximately, in the time required to reach the peak of the curve, the relative amount of mixing the sample will need in the baking test if an optimum mix is to be employed. In baking the present series of samples a tendency towards shorter time with increasing protein content was noted for the optimum mixing time method. From the curves it may be observed that less time was also required to attain optimum development (peak of curve) in the higher-protein samples.

Examination of the curves shows that the type or pattern changes somewhat with the variation of protein within a variety but the pattern differences between varieties are greater than within a variety. Within a variety the pattern is more or less characteristic although some varieties tend to resemble others of similar dough-handling properties. Early Blackhull and Chiefkan are fairly similar and both differ from Blackhull, which possesses better handling properties. Blackhull curves, however, differ to a considerable extent from either Tenmarq or Turkey. The curve pattern of Kawvale resembles Turkey much more closely than either of the soft winter varieties, Michigan Wonder and Clarkan. In the trade, Kawvale has proved acceptable for family trade flours but is definitely not suitable for high-quality soft-wheat flours used for either cake or soda cracker production.

Examination of the curves inevitably raises the question as to which type is to be considered most desirable. Such a decision is entirely dependent upon the market use of the flour. Among Kansas mills, Turkey

is widely favored and flours with the characteristics of Turkey find ready acceptance in the baking trade. Any variety that does not differ too widely in characteristics from Turkey should therefore also prove satisfactory. Permissible deviation from a standard without causing consumer complaint will depend upon the degree of specialization and standardization in the consuming market. A bakery accustomed to Blackhull type of flour may easily object to Tenmarq flour as being too strong. On the other hand, if Turkey were the accustomed standard flour, then Kanred might be substituted without complaint although Cheyenne might be objectionable because of its excessively long mixing requirement. The difference between Clarkan and Michigan Wonder may be so much that the two varieties cannot be readily interchanged. Many users of soft-wheat flour require a good quality of gluten, even though in small amount, rather than a larger amount of poorer gluten. Additional information is needed as to the desirable curve type for the various specialized purposes for which soft-wheat flours are used.

Summary

Winter wheat of several varieties grown at various places in Kansas was so composited that for each variety a series of samples differing in protein level became available.

Each sample was milled and the flour subjected to two baking tests which differed only in that one method employed optimum mixing time and the other a fixed mixing period of three minutes. The rankings of the varieties from the two baking methods were not identical. The differences were most evident at the lower protein levels. It is considered that these data are not conclusive as to whether an optimum mixing time is essential for the satisfactory evaluation of wheat varieties.

The data indicate the existence of a linear relationship between protein content and loaf volume. This is more obvious in some varieties than in others.

Protein-loaf volume regression lines indicate that these varieties differ in gluten quality. Some varieties are relatively poorer or relatively better in protein quality than others at equal protein levels.

Micro-mixer curves with optimum baking absorptions, indicating dough-handling properties, show that large differences in these properties exist among the varieties studied. These differences are large enough so that some of the varieties probably could not be used interchangeably without causing serious trouble in commercial bakeshops.

Chiefkan and Cheyenne gave the poorest baking results, as indicated by smaller loaf volume, among the hard wheat varieties.

The high baking quality and the curve type of Kawvale show that its quality *as a soft wheat* is poor. This variety grades red winter on the market.

Clarkan and Michigan Wonder differ materially in baking performance and curve type although both are graded as soft winter varieties.

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FOREIGN MATTER IN CORN MEAL

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Cereals contaminated with rodent excreta or by insect infestation are unfit for food. A product such as corn meal may be contaminated because it has been made from filthy corn, or because an infestation has developed in the meal itself. Insects in whole corn will be broken to bits when the corn is ground but the meal is still contaminated with their fragments and their excreta. The same is true of grain containing rodent excreta. Grinding does not remove the excreta. It changes only their shape and makes both insects and excreta less readily detectable by a casual examination.

Some time ago it became evident that corn meal might contain such ground-up filth, and a method was devised to detect the contamination. This procedure is based on a principle employed in other filth-extraction methods issued by the Food and Drug Administration, certain details of which have been specifically modified to insure a clean flotation of insects and rodent hairs with many types of corn meal. In addition, there has been inserted a procedure for the sedimentation of heavy filth. The removal of rodent and insect contamination involves a separation based on the unequal density of the food and filth particles and the affinity of insects and animal hairs to oils.

Many insect excreta are heavier than the cereals, but on the other hand, there are light excreta pellets and some heavier cereal tissues which render a specific gravity separation impractical. When flour is cleared in oil, the pellets, which remain opaque, stand out by contrast. Rodent-excreta-pellet fragments are generally heavier than the comminuted cereals; in a liquid with a specific gravity near 1.49 they will tend to settle out while much of the cereal floats. As the specific gravity is raised, more cereal is floated, but some pellet fragments will also rise and be lost. It is necessary to strike a practical balance between the need to float the plant tissue and the possible loss of excreta. Factors other than density play a part in this separation. The particles must be soaked in the liquid to give them time to become fully permeated. To work the excreta loose from the cereal tissue, the mixtures must be stirred. The density balance is quite delicate and beakers should be covered and otherwise handled to avoid strong convection currents. Rather than attempting to complete the separation with one decantation, it should be repeated as may be necessary. During these decantings, one

should keep the beakers tilted on edge. In this manner the heavy filth will be in one mass, easily watched and controlled.

Insects, insect fragments, and rodent hairs are often lighter than the cereal and sometimes may be floated out in heavier-than-water liquids while the plant tissue settles out. Usually, however, they are extracted by a different procedure. With the exception of fly larvae, or maggots, insects and insect fragments can be wet with oils mixed into an aqueous mixture of a food and so floated up to the surface with the oil. In practice, because of several factors, this separation is not complete. It is difficult to wet all of the insect material without creating a frothy emulsion of the plant material that will obscure the filth particles in the subsequent microscopic examination. Fragments may become trapped in or attached to a mass of plant material settling out. Droplets of oil often adhere to the sides of the trap flask and may hold insects there, thus keeping them from rising. To reduce some of these effects, the oil or gasoline is worked thoroughly into the water-cereal mixture, but with no "whipping" and as little inclusion of air as possible. Intermittent agitation is provided while the separation is taking place. Some products cannot be extracted in water because too much of the food rises with the "light filth." To reduce some of these effects, the oil or gasoline is worked thoroughly into the water-cereal mixture, but with no "whipping" and as little inclusion of air as possible. Intermittent agitation is provided while the separation is taking place. Some products cannot be extracted in water because too much of the food rises with the "light filth." To reduce floury emulsions, the extractions can be made in saturated salt solution. Sometimes caprylic alcohol or 95% ethyl alcohol can be used to break an emulsion. In general, when much bran or chaff is present, it will float up with the oil when water or saturated salt is used and it is advisable to do the extracting in a water-ethanol solution. (For some cereals a water-isopropyl alcohol solution may be used.) The alcohol not only soaks into bran but it is also less dense, so that less plant tissue floats. Material trapped off in one Wildman trap [1] may be transferred to another trap and rewashed to remove some of the plant material. While this is necessary in many instances, any nonessential operations may lose filth material and are to be avoided.

Filter papers should be so treated that the microscopic examination is as simple as possible. To accomplish this it is advisable to (1) clear any extraneous plant material that may be present, (2) prepare excreta for examination, and (3) use coarse filters that have been marked off into areas to facilitate examination. Flour and bran may be cleared with mineral oil or chloral hydrate, thus rendering the insect fragments and

[1] First described by B. J. Howard in *Food Industries*, July, 1935.

rodent hairs more readily visible. If mineral oil is used, the material on the filter must be air-dried before the oil is added. Mineral oil is well suited to the microscopic determination of insects, insect fragments, and rodent hairs, but in it rodent excreta become so hard it is difficult to open the excreta to find the rodent hairs. Hence the sediment from a chloroform extraction should be cleared and softened in 75% alcohol. If an excessive amount of starchy material is present, the paper may be completely cleared by gelatinizing it with chloral hydrate. The chloral leaves the pellet fragments soft but is extremely noxious to work with and should be washed out of the filter after the clearing is complete and before the microscopic examination is made.

The filter paper should be ruled in fine parallel lines 6 to 7 mm apart. Filter paper may be purchased with water-, alcohol-, and oil-proof lines printed on it or the lines may be applied conveniently by means of a rubber stamp and pad. Waterproof India ink makes a permanent nonspreading line. If the filter paper is not ruled, it is necessary to place a wire grid over the paper to mark it off into smaller areas.

In testing corn meal, care should be taken to secure representative samples.[2] They should be fumigated at once with chloroform or carbon disulfide vapor or held under refrigeration to prevent any living insects from working in the sample.

Procedure

Separation of rodent excreta by sedimentation: Weigh out a 50-g portion of the well-mixed sample into a 250-ml hooked-lip beaker. Add chloroform to within ½ inch of the top, mix thoroughly and allow to settle for at least 30 minutes. Several times during this period stir the layer that rises to the top. Decant the chloroform and the floating corn tissue into a 7-cm Buchner funnel attached to a suction flask and containing a smooth-surface filter paper, being careful not to disturb the heavy residue in the bottom of the beaker. Before decanting, take care that the floating layer has not become so compact that this operation is difficult. Keeping the beaker tilted on edge, add chloroform, allow to stand for several minutes, and again decant. Add carbon tetrachloride in an amount equal to the chloroform and corn tissue left in the beaker, allow to settle again, and decant as before. Repeat this process with a mixture of equal parts of chloroform and carbon tetrachloride until very little corn tissue is left in the beaker. Avoid decanting any rodent excreta fragments that may be present. At this point the bulk of the corn meal will be in the Buchner funnel and the heavy residue, including the rodent excreta fragments, if any, will remain in the beaker.

[2] W. G. Helsel and Kenton L. Harris, Method for the Recovery of Filth from Corn Meal (Tentative), U. S. Food and Drug Administration. Mimeographed. Revised December 12, 1939.

The residue in the beaker is now washed onto a 7-cm filter paper with a stream of chloroform or carbon tetrachloride. Carefully transfer the filter paper to a petri dish. Immediately before the microscopic examination is made, add sufficient 70% alcohol to wet the filter paper and material on it thoroughly while not causing the corn and filth particles to flow. This alcohol will soften the rodent excreta pellet fragments and clear the corn tissue.

Separation of rodent hairs and insects by flotation: Draw air through the Buchner funnel until the chloroform-carbon-tetrachloride mixture is evaporated. Carefully transfer the contents of the funnel onto a large sheet of clean, smooth paper. If the material is clumped, dry off the remaining chloroform in the air or in an oven at 60°–65°C. Transfer this material to a one-liter Wildman trap flask. Add 100 ml of 60% ethyl alcohol or 53% isopropyl alcohol (by volume) and mix thoroughly. (This alcohol is used with the usual type of whole corn meal, which may or may not have been sifted and/or bolted. In case degerminated or cream meal is being tested, or meal that has had the bran and chaff removed by bolting and/or aspiration, the same procedure is followed except that distilled water or a saturated salt solution is used instead of the 60% alcohol, and only 25 ml of gasoline is required.) Wash down the sides of the flask with a stream of 60% alcohol from a wash bottle and allow the material to soak for 30 minutes. Add 35–40 ml of gasoline, mix thoroughly, and allow to stand for 5 minutes. While stirring, add more 60% alcohol until the floating gasoline layer carrying the filth and some corn tissue can be trapped off in the neck of the flask. Allow to stand for 30 minutes, stirring 4 to 6 times during this period in order to release filth fragments trapped in the bottom of the flask, and also to permit some of the corn tissue to settle out of the neck of the flask. (If a large amount of emulsion has formed in the neck, it may be broken with a very fine stream of 95% alcohol from a wash bottle.) After a few minutes, raise the stopper into the neck of the flask and by gentle agitation release any corn tissue that has begun to settle out. The gasoline layer must be allowed to separate completely again before it is trapped off. Carefully spin the stopper to remove corn meal from its top and trap off the filth by drawing the stopper tightly into the neck of the flask. The gasoline and about ⅓ to ½ inch of the alcohol layer should be trapped off above the stopper and filtered through a rapid-acting filter paper in a Buchner funnel.

Rinse the contents of the neck of the flask carefully into the funnel—first with alcohol and then with water. Wash down the sides of the Buchner funnel with a stream of alcohol and continue suction until the paper is dry. Hold the funnel tilted against the edge of a petri dish

containing a few drops of mineral oil and carefully remove the filter paper to the dish. This oil serves to clear the corn tissue, making the filth fragments more readily visible. Add 20 ml of gasoline to the material in the flask and stir vigorously to draw the gasoline down into the mixture. Add sufficient 60% alcohol to bring the gasoline layer well into the neck of the flask. (If distilled water or salt solution was used in the first extraction it should be substituted here.) Allow to stand for 10 minutes before again trapping off into a Buchner funnel and transferring to a petri dish.

Identification and recording of filth: After clearing, the material on the filter papers is ready for examination with a Greenough type binocular dissecting microscope using 20 to 30 magnifications. A white background and reflected light should be employed. The following may be recovered from the residue left after the chloroform-carbon-tetrachloride decanting: rodent excreta fragments (identified by the presence of pieces of rodent hairs), a few insect fragments (especially those embedded in rodent or insect feces), a small number of hairs, and nondescript filth fragments. From the gasoline extraction may be recovered rodent hair fragments, a few hairs with pieces of excreta attached, insects, and insect fragments. If there is any doubt about the identification of any of the fragments, they should be removed to a slide and examined under the compound microscope.

Count and record the findings as follows:

Rodent-excreta-pellet fragments
Detached rodent hair fragments
Adult insects
Pupae
Detached adult insect heads
Insect larvae
Cast skins and capsules
Detached insect larvae heads
Miscellaneous insect fragments
Nondescript filth fragments

It is essential that the analyst be able to recognize the extracted filth, and proficiency in this direction can be gained only through an examination of authentic samples. Various food-infesting insects should be collected and studied. To learn what they look like in comminuted products, individual insects should be carefully dissected *in detail* and the various parts studied at 20 to 30 magnifications so that their fragments can be recognized. One of the references given later may be helpful as a guide in this work. Analyses should not be attempted until several

species have been worked over. Insects develop in four stages, the egg, larva, pupa, and adult bearing little resemblance to each other. Each stage should be separately studied. It is suggested that the confused flour beetle, saw-toothed grain beetle, granary weevils, Angoumois grain moth or Indian meal moth, meal worms, and mites be the main objects of study. Little or no attention need be paid to the internal anatomy.

Similarly, rodent and other types of hairs should be examined. Hairs embedded in excreta and in fragments of excreta should be compared with material of known origin. Insect excreta may be readily obtained from some of the more common storage insects which may be reared conveniently in the laboratory. Types from various kinds of insects should be examined.

The resolving power of the compound microscope is superior to that of the Greenough type binocular and during the beginning work it should be used frequently at 100 to 200 magnifications to study details of structure. As progress is made, it may be used with less frequency.

After whole and dissected insects and rodent filth have been studied, the same types may be studied after being ground in a mortar. Authentic material must be used. A study may then be made of tissues of corn and wheat. (See the books by Winton referred to later.) The kernels should be examined with the naked eye and then studied as carefully as were the contaminants. At this point, differences between the plant and insect tissues should be noted.

After this work has been completed, the methods for extracting filth from foods should be studied. By practicing with grossly contaminated samples, it is possible to develop the manual technique and, at the same time, extract much material for subsequent microscopic observation. This comparative work must be continually checked back to authentic material.

Obviously the technician should be thoroughly familiar with the proper use of the microscope in the identification of filth. One of the best books available for this purpose is *The Microscope*, by S. H. Gage (Comstock Publishing Co., Ithaca, N. Y.), 1932. For identifying plant structures the *Textbook of General Botany*, by R. M. Holman and W. W. Robbins, 1939, is suitable, but for the details of seed structure, books such as the following may be consulted: *The Microscopy of Vegetable Foods*, by A. L. Winton, 1916, and *The Structure and Composition of Foods*, Vol. I, by Winton and Winton, 1932. The latter three books are published by John Wiley and Sons, New York.

Often it will be desirable to use some references in studying the contaminants. For guidance in the insect study, *An Introduction to Entomology*, by J. H. Comstock (Comstock Publishing Co., Ithaca, N. Y.), 1920, may be used. *Principles of Insect Morphology*, by R. E. Snod-

grass (McGraw-Hill Book Co., N. Y.), 1935, is recommended only as a detailed, highly technical reference book. There are several excellent volumes that can be used as a general guide to the classification of insects, but the U. S. Department of Agriculture Farmers' Bulletin 1260, *Stored Grain Pests,* 1938, probably will be sufficiently complete for general use. U. S. Department of Agriculture Miscellaneous Publication 318, *4–H Club Insect Manual,* 1940, may be helpful, and Miscellaneous Publication 258, *Annotated List of the Insects and Mites Associated with Stored Grain and Cereal Products,* 1937, gives a more complete list of references. Both these and the following references on hairs are amply illustrated.

The microscopic characteristics of animal hairs are discussed in several publications. *A Study of Hairs and Wool,* by John Glaister, Misr Press, Cairo, Egypt, 1931, contains an extensive discussion of the structural characteristics of the hairs of many mammals including rodents, cats, dogs, and human beings. The *Journal of Wildlife Management,* Vol. II, pp. 239–250, 1938, contains an article on mole and shrew hairs and a concise review of the microscopical features of hairs and hair types. For a description of some common hairs and the methods of studying them, there is an article in the February 21, 1920, *Scientific American,* page 200, by L. A. Hausman. Similar material concerning fur hairs appears in an article by the same author in the January, 1920, *Scientific Monthly,* page 70, while more complete data on a variety of mammals appear in his excellent *American Naturalist* article, " Structural Characteristics of the Hair of Mammals," page 496, 1920. Some further details of hair structure, perhaps of interest to the research worker, appear in the *American Naturalist* for November–December, 1924, page 544.

THE DETERMINATION OF NICOTINIC ACID IN BREAD AND OTHER CEREAL PRODUCTS

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Since the discovery by Elvehjem and coworkers of Wisconsin in 1937 that nicotinic acid is a vitamin and specific for the prevention and cure of pellagra, there has been a demand for a rapid, accurate method for the determination of nicotinic acid applicable to bread and cereal products.

Bandier and Hald (1939) found that metol (*p*-methyl amino phenol sulfate) plus nicotinic acid and cyanogen bromide in aqueous solution

yields a clear yellow color which is perfectly stable for a period sufficient to be read, and of an intensity directly proportional to the amount of nicotinic acid. We tried this method and found it applicable to the determination of nicotinic acid in yeast if extreme caution is observed, but not applicable to cereal products like flour and bread. In determining nicotinic acid in products to which the method is applicable we found that even minute residual quantities of acetone used in the process vitiated the results and that it was very difficult to remove the last traces of this solvent. The color technique employed was not satisfactory because of the high blank values and turbidity, which introduced a source of error.

In the determination of nicotinic acid in yeast by the Bandier and Hald method reasonably accurate results were obtained, but in many instances the values were erratic. When 0.66 μg of nicotinic acid per gram was added to yeast, 0.69 μg per gram of yeast was found. Since yeast also contains nicotinic acid, the difference represents about the normal amount contained in the yeast before the addition. When this method is applied to flour, bread, and other cereal products a semisolid mass results which will not produce an extract suitable for subsequent analytical treatment.

It occurred to us that this semisolid mass could be liquefied by the application of diastase, since the treatment given was sufficient to prepare the starchy material for diastasis. This proved true and we found that any suitable diastatic reagent could be used for the purpose. We employed takadiastase and found it quite suitable for the liquefaction. Since takadiastase is also used in the Hennessy method for the determination of thiamin, we thought its use as the diastatic reagent in our process would avoid the introduction of another reagent.

The subsequent treatment of the sample by the Bandier and Hald procedure, where acetone is employed, produced turbidity and high blank values that proved unsatisfactory for cereal products after treatment with diastase.

The method of Arnold, Schreffler, and Lipsius (1940) for the determination of nicotinic acid is not applicable to flour, bread, and cereal products, but their color production technique for the treatment of our liquefied extract from diastase was found applicable for the reading of the solutions. These authors used *p*-aminoacetophenone and cyanogen bromide to produce a stable color that gives a relatively low extinction coefficient in the blank, free from turbidity and other interfering sources of error.

Experimental

Preparation of extract: A sample of the bread to be assayed for nicotinic acid is sliced and preferably dried in a hot-air oven (80°–90°C) overnight.[1] A moisture determination is made so that the bread can be calculated to a definite moisture content. The dried bread is reduced to fine particle size with mortar and pestle or is ground. Twelve grams are placed in a centrifuge bottle and suspended in 75 ml of distilled water. The sample is then autoclaved for 10 to 15 minutes at 15 pounds pressure. After cooling to 50°–60°C, 0.3 g of takadiastase is added and held at 50°–60°C for one hour, centrifuged, and the supernatant fluid decanted into a 100-ml graduate. The residue is shaken up with 30 ml of distilled water, centrifuged and added to the first extract. The total liquid of about 80-ml volume is transferred to a 125-ml Erlenmeyer flask, 5 ml of concentrated HCl added, and the extract heated for 30 minutes on a boiling water bath. After cooling, 20% NaOH is added to bring the pH to approximately 4. After standing for 5 minutes the liquid is transferred to a centrifuge bottle and centrifuged at high speed for 10 minutes. The clear solution is then brought to a pH of 6 with 20% NaOH and transferred to a 100-ml graduate. The volume of 85–90 ml is accurately noted, as this volume measurement is used in computing the nicotinic acid concentration of the entire sample.

Color production: If the original moist bread contains around 10 mg of nicotinic acid per pound, 5-ml portions are measured into each of the four 15-ml amber glass graduated cylinders; if only about 5 mg of nicotinic acid is contained per pound, use 8- to 10-ml aliquot portions. Twenty micrograms of nicotinic acid are added to one of the flasks and 40 to another. All four cylinders are then heated in a hot water bath (80°C) for 10 minutes and then 2 ml of cyanogen bromide added (saturated bromine water just decolorized with 10% KCN in the cold) to three of the cylinders, including the two cylinders with added nicotinic acid. After heating an additional 4 minutes at 80° C, the cylinders are cooled rapidly to room temperature, and after 4 minutes 0.2 ml *p*-aminoacetophenone is added to each cylinder (10 g dissolved in 28 ml of 10% HCl diluted to 100 ml). The graduates are shaken, placed in the dark for 15 minutes, and then 0.4 ml of 10% HCl is added from a microburette, after which they are allowed to stand 15 minutes more in the dark. The volume of each graduate is made up to 13 ml with distilled water. The mixtures are then transferred to a 25-ml centrifuge separatory funnel (Pfaltz & Bauer, Inc.) containing 15 ml of ethyl acetate. The funnels are shaken for exactly 5 minutes and the water layer is then

[1] The bread sample does not have to be dried for the determination, but only to give more accurate data for the calculation and to give a higher concentration of nicotinic acid per unit weight of bread.

drawn off and discarded. The ethyl acetate is clarified with 2 g of anhydrous Na_2SO_4 whereupon it is ready for colorimetric reading. A Pfaltz & Bauer fluorophotometer is used with a combination blue and yellow filter to give a wave length of about 420 μ. The light intensity is adjusted to read zero extinction with ethyl acetate with switch in transmission position. The extinction value of the sample is then determined. The blank reading is subtracted from the other values obtained. The corrected values are then plotted on graph paper and the value of the nicotinic acid in the original sample thus obtained (Fig. 1).

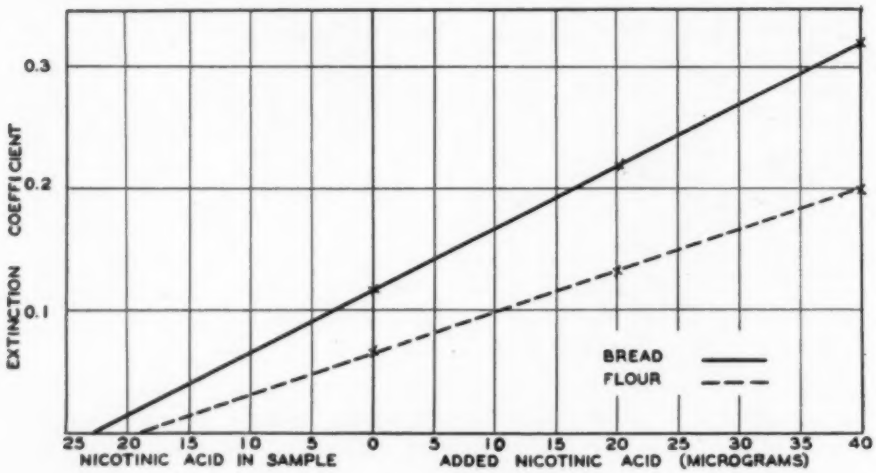

Fig. 1. Data used in the nicotinic acid method.

Bread data:		Flour data:	
Weight dried bread used	12.0 g	Weight flour used	10.0 g
Volume of extract	85.0 ml	Volume of extract	100.0 ml
Volume of extract used	5.0 ml	Volume of extract used	10.0 ml
Bread moisture	34.0 %	Nicotinic acid superimposed	10 μg
Nicotinic acid content	9.75 mg/lb	Nicotinic acid content	3.85 mg/lb

Comparative data (basis 1 lb bread):

	Nicotinic acid in Yeast	Bread	Flour (diff.)
(1)	7.55 mg	9.75 mg	2.20 mg
(2)	6.05 mg	8.60 mg	2.55 mg

Nicotinic acid in flour by assay ..... 3.85 mg/lb
Nicotinic acid in bread (calculated) ..... 2.42 mg/lb

Galvanometer readings:

Bread		(Corrected)	Flour		(Corrected)
B	0.034		B	0.035	
0	0.149	0.115	0	0.101	0.066
20	0.254	0.22	20	0.166	0.131
40	0.352	0.318	40	0.234	0.199

Recovery of added nicotinic acid in bread: Samples of bread used in this experiment were divided into two groups. Group A had 5 mg of added nicotinic acid per pound of moist bread. Group B had 10 mg of

added nicotinic acid per pound of moist bread. The following results were obtained:

Sample No.	Group A *mg nicotinic acid per lb moist bread*
1	5.53
2	5.40
3	5.36
4	5.48
5	5.42

Sample No.	Group B *mg nicotinic acid per lb moist bread*
1	10.85
2	10.90
3	10.76
4	10.83
5	10.79

Determination of nicotinic acid in flour: The determination of nicotinic acid in flour is best carried out by adding a portion, 0.2 g, of takadiastase to the flour sample and heating to 65° to 70°C with stirring; holding at this temperature for 15 minutes, then bringing to a boil and pressure cooking to avoid clumping. The sample is then cooled to 50°–60°C, 0.3 g more takadiastase added, and the liquefaction completed. The procedure from here on is the same as for bread, except 10-ml portions of the extract are used for color development with 10 μg of nicotinic acid superimposed in each portion in addition to the samples containing the added 20 and 40 μg of nicotinic acid. This modification brings the readings up into the desired range.

The method given in this paper appears to be simpler in many details than that proposed by Melnick, Oser, and Siegel at the spring meeting of the American Chemical Society (1941). The color production technique of Arnold, Schreffler, and Lipsius (1940) was used because of the relatively low extinction values obtained in the blanks. Bandier-Hald's color technique was tried but rejected because of the high blank values which introduced a source of error.

Reagents and Apparatus

Reagents:

1. Cyanogen bromide: saturated bromine water just decolorized with 10% potassium cyanide in the cold.
2. *p*-aminoacetophenone: 10 g dissolved in 28 ml of 10% HCl and made up to 100 ml.
3. Ethyl acetate, reagent grade.
4. Sodium sulfate, anhydrous, reagent grade.

Apparatus:

1. Centrifuge bottles: 200–230 ml.
2. Amber glass graduates: 15 ml (Otto R. Greiner Co., Newark, N. J.).
3. Centrifuge separatory funnels: 25 ml (Pfaltz and Bauer, Inc., New York).
4. Fluorophotometer: (Pfaltz & Bauer, Inc., New York).

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THIAMIN CONTENT OF TYPICAL SOFT WHEAT MILL STREAMS

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Within the last few years the determination of thiamin (vitamin B_1) in cereals has presented one of the outstanding problems to the cereal chemist. The oxidation of thiamin to thiochrome and the subsequent quantitative measurement of this fluorescent compound has been utilized by a number of workers with varying degrees of success. Jansen (1936) first devised a procedure for thiamin by this method using quinine sulfate as a standard of fluorescence. Karrer and Kubli (1937) used a pure solution of thiamin oxidized in parallel with the unknown as a standard. Westenbrink and Goudsmit (1937) first removed thiamin from interfering substances by adsorption on franconite and used the method for analysis of urine. Various other modifications have been introduced by Pyke (1937) and Hennessy and Cerecedo (1939). Wang and Harris (1939) encountered thiamin losses in the absorption and elution with zeolite. They removed soluble interfering substances by extracting the thiamin solution with isobutyl alcohol. Booth (1940) used a modified Wang and Harris method on a number of English wheats. Schultz, Atkin, and Frey (1939) have reported the thiamin content of wheat, various grades of flour, bran, shorts, and germ, measured by the yeast fermentation test.

Experimental

Believing that a definite contribution could be made to the knowledge of thiamin in cereals by a determination of the thiamin content of various mill streams, we obtained from a mill employing a five-break, seven-reduction system, representative samples of the flours produced together with typical samples of red dog, shorts, and bran. All samples were milled from the same sample of commercial Ohio soft wheat.

After a preliminary survey of methods the following procedure was adopted from the method of Wang and Harris. Two- to five-gram samples were weighed out into 125-ml Erlenmeyer flasks, and 50 ml of 2% acetic acid was then added and mixed with the sample. The mixture was placed in a boiling water bath for 15 minutes, allowed to cool, and then 5 ml of 1*N* sodium hydroxide was added and mixed well. This was followed by 5 ml of a solution of takadiastase containing 4 g of takadiastase per 100 ml. The mixture was incubated at 42°–45°C for 90–100 minutes and then centrifuged until the supernatant fluid was clear.

The clear solution was decanted off into a cylinder containing 20 ml of isobutyl alcohol and shaken vigorously for one minute. The alcoholic and aqueous layers were allowed to separate by gravity or by centrifuging. The solutions were then poured carefully into a separatory funnel, the lower aqueous layer drawn off, and the alcoholic layer discarded.

Three aliquots of the aqueous extract were taken for oxidation. The three aliquots were placed in 125-ml separatory funnels. One-tenth to 0.25 ml of 1% potassium ferricyanide (depending upon the thiamin concentration) was added and mixed, followed by 3 ml of 15% sodium hydroxide and immediately 15 ml of isobutyl alcohol, with mixing after each addition. The samples were then vigorously shaken for 1½ minutes and allowed to stand until the layers separated. The third aliquot was used as a blank. It was treated like the other two except that the potassium ferricyanide was omitted and the alcohol was added before the sodium hydroxide.

After the two layers had separated, the aqueous layer was drawn off and discarded. The isobutyl layer containing the thiochrome was run into a test tube and 1 ml of 95% ethyl alcohol added to clarify it. The clear solution was compared in a Pfaltz and Bauer fluorometer, with a quinine sulfate solution used as a standard. This standard was checked daily against a solution containing one microgram of thiamin which was oxidized by the above procedure just previous to comparison. This method was found to check closely with the method of Hennessy and Cerecedo (1939) using zeolite. The thiamin content was calculated by

the equation:

$$\mu g/g = Dx/Ds \times 60/A \times 1/S$$

Where: Dx = galvanometer deflection of unknown
Ds = deflection of one microgram standard of thiamin
A = ml in aliquot
S = weight of sample in grams
60 = volume sample extract

Amounts of thiamin in the various mill streams are given in Table I. Accuracy of the method was determined by a recovery test made by adding a known amount of thiamin to a sample previously analyzed and repeating the determination on the reinforced sample. Since all the

TABLE I

DISTRIBUTION OF THIAMIN IN THE VARIOUS MILL STREAMS

Laboratory number	Stream	Thiamin (μg per g)		Ash content, 13.5% moisture basis
		Found [1]	Corrected (times 1.11)	
				%
1179	1st break flour	0.8	0.9	0.404
1180	2nd break flour	1.0	1.1	0.406
1181	3rd break flour	0.8	0.9	0.474
1182	4th break flour	0.7	0.8	0.480
1183	5th break flour	1.3	1.4	0.590
1184	1st mids flour	0.9	1.0	0.338
1185	2nd mids flour	0.6	0.7	0.350
1186	4th mids flour	0.8	0.9	0.376
1187	5th mids flour	1.3	1.4	0.424
1188	6th mids flour	2.0	2.2	0.478
1189	7th mids flour	2.4	2.6	0.648
1190	1st tailings flour	1.1	1.2	0.535
1191	2nd tailings flour	1.8	2.0	0.560
1192	Sizings flour	0.7	0.8	0.326
1193	Bran and shorts dust reel	2.4	2.6	0.740
1194	1, 2, 3 breaks, hex. reel cuts	1.2	1.3	0.840
1195	Low-grade flour	3.6	3.9	0.684
1196	3rd mids patent flour	0.7	0.8	0.328
1197	3rd mids clear flour	0.9	1.0	0.358
1198	Red dog	9.2	10.1	—
1199	Shorts	9.3	10.2	—
1200	Bran	5.2	5.7	—

[1] At least two analyses on different days, checking within 10% of each other, were used in obtaining the average results.

percentage recoveries, shown in Table II, on all samples investigated were from 88% to 91% it was believed safe to multiply each result by the factor 1.11 to get the true thiamin value.

Some work was done on the necessity for hydrolyzing possible phosphate complexes of thiamin with takadiastase. If little or no phosphory-

TABLE II

RECOVERY OF ADDED THIAMIN

Laboratory number	Stream	Micrograms of thiamin			Recovery	
		In sample	Added	Found	Micrograms	%
1179	1st break flour	4.0	20	21.6	17.6	88
1183	5th break flour	6.5	20	24.2	17.7	89
1190	1st tails flour	5.5	20	23.7	18.2	91
1192	Sizings flour	3.5	20	21.1	17.6	88
1195	Low-grade flour	10.8	20	29.0	18.2	91
1197	3rd mids clear flour	4.5	20	22.5	18.0	90
1198	Red dog	18.4	20	36.2	17.8	89
1199	Shorts	18.6	20	36.2	17.6	88

lated thiamin occurs in cereal products this step could be omitted with much saving of time. The procedure used was the same as previously outlined except that the addition of 1*N* sodium hydroxide and takadiastase and the incubation at 45° were omitted. Results are given in Table III.

TABLE III

EFFECT OF TREATMENT WITH TAKADIASTASE

Laboratory number	Stream	Thiamin (μg per g)	
		With takadiastase (uncorrected)	Without takadiastase (uncorrected)
1180	2nd break flour	1.0	1.0
1181	3rd break flour	0.8	1.0
1182	4th break flour	0.7	0.7
1184	1st mids flour	0.9	0.8
1185	2nd mids flour	0.6	0.6
1190	1st tails flour	1.1	1.0
1191	2nd tails flour	1.8	1.5
1192	Sizings flour	0.7	0.6
1195	Low-grade flour	3.6	3.66
1196	3rd mids patent flour	0.7	0.7
1198	Red dog	9.2	9.0
1199	Shorts	9.3	5.8

Apparently the use of takadiastase is unnecessary, at least in flour, although more work should be done on this phase.

Pepsin digestion was investigated with bran, but without increasing the thiamin value, although the blank was higher in the case where pepsin was used, as follows (bran 3-gram sample, 60-ml extract, 5-ml aliquot): *With pepsin:* Reading thiochrome 45, blank 18, net 27. *Without pepsin:* Reading thiochrome 39, blank 13, net 26.

Conclusions

The thiamin content of mill streams from the same sample of Ohio wheat varied widely. In a general way there seems to be a direct relationship between ash content and thiamin content. This is in agreement with the work of Hoffman, Schweitzer, and Dalby (1940), who found a definite relationship between thiamin content and ash content in clear flours.

The method of Hennessy can be shortened for wheat products with no loss of accuracy by the substitution of a preliminary extraction of the unknown solution with isobutyl alcohol for the adsorption on zeolite. It is doubtful that the use of takadiastase is necessary for the analysis of thiamin in wheat flour.

Summary

A number of flours of different mill streams with the bran, shorts, and red dog milled from the same sample of wheat were analyzed for thiamin by a modified thiochrome method. The thiamin content of the flours varied between 0.7–3.9 μg per gram, following roughly their respective ash contents.

It is suggested that takadiastase may be unnecessary in the analytical procedure. Red dog flour contained 10.1 μg per gram, while shorts, which probably contained much of the germ as well as fine bran, contained 10.2 μg per gram and the bran contained 5.7 μg per gram.

Since bran contained about the same concentration of thiamin as whole wheat, it can be left out of bread flour without appreciable sacrifice of thiamin. The shorts and red dog fractions, however, are considerably higher in thiamin than the original wheat; hence their removal causes large losses in the vitamin. The low-grade flours have slightly lower thiamin values than the whole wheat from which they originated.

Acknowledgment

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THE THIAMIN AND RIBOFLAVIN CONTENTS OF WHEAT AND CORN[1]

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The chemical methods for the determination of riboflavin and thiamin which have recently been developed make possible a more comprehensive study of the occurrence of these vitamins in wheat and corn than would be feasible with the tedious and costly biological methods. Such a study is timely as a result of the current interest in vitamin enrichment of cereal products to whole-grain levels. In order to carry out intelligently the fortification of cereal products, it is necessary to know the variations in the quantities of the vitamins in the whole grain, and the factors influencing these variations. The authors have investigated some of these factors, using the procedure described in a previous paper (1941) for the combined determination of thiamin and riboflavin.

Because of limitations inherent in biological methods, many of the values found in the literature for the thiamin and riboflavin content of wheat and corn were necessarily based upon assays of small numbers of samples. Consequently these data do not indicate the range of vitamin values that may occur.

Chemical analyses of wheat and corn for thiamin have been reported recently, but as far as the authors are aware, no such study has been reported for the riboflavin content of these grains. Schultz, Atkin, and Frey (1939, 1941), using the yeast fermentation procedure, reported

[1] Presented at the American Chemical Society Meeting at St. Louis on April 9, 1941.

thiamin values for wheat samples varying from 4.2 to 7.3 μg per gram, with an average value of 5.6. Hoffman, Schweitzer, and Dalby (1940), using the same procedure, analyzed 46 samples of whole wheat flour derived from wheat of different varieties and from various localities. They found the average thiamin content to be 6.85 μg per gram. Booth (1940), employing the thiochrome method, analyzed 78 varieties of wheat, common to the English market. He has reported values ranging from 1.62 to 9.99 μg of thiamin per gram with an average value of 3.75. A sample of durum wheat had the highest thiamin content, and English spring wheats were generally richer in thiamin than the winter varieties. As Booth's method did not entirely eliminate interfering substances, his figures may be expected to be slightly high.

Riboflavin and Thiamin Contents of Hard and Soft Wheat

Fifteen varieties of hard wheat and 16 varieties of soft wheat were analyzed for thiamin and riboflavin.[2] The values obtained are shown in Tables I and II.

TABLE I

RIBOFLAVIN AND THIAMIN CONTENT OF VARIETIES OF HARD WHEAT

Variety	Thiamin	Riboflavin
	μg/g	μg/g
Amber Durum	5.80	1.20
Blackhull	5.84	1.09
Ceres	4.35	1.06
Cheyenne	4.88	1.09
Chiefkan	4.57	1.14
Early Baart	6.90	1.31
Kanred	5.20	1.41
Marquis	4.35	1.31
Montana Marquis	4.98	0.96
Nebraska No. 60	4.00	1.91
Nebred	4.98	1.27
Ridit	3.65	0.94
Tenmarq	5.30	1.03
Turkey	5.72	1.16
Turkey Red	6.05	0.89

The thiamin content of the hard wheat varieties shown in Table I range from 3.65 to 6.90 μg per gram, with an average value of 5.03. The values given in Table II for the soft wheats vary from 2.43 to 4.77 μg of thiamin per gram with an average of 3.52. The results shown in Tables I and II are in confirmation of Booth's (1940) conclusion that hard wheats have a somewhat higher thiamin content than the soft-

[2] The authors are indebted to Mr. C. C. Fifield of the Bureau of Plant Industry of the U. S. Department of Agriculture for his kind cooperation toward securing many of the wheat samples used in this study.

TABLE II

RIBOFLAVIN AND THIAMIN CONTENT OF VARIETIES OF SOFT WHEAT

Variety	Thiamin	Riboflavin
	μg/g	μg/g
Albit	3.91	0.89
Currell	3.25	1.48
Dicklow	3.63	1.24
Federation	3.12	0.81
Fulhio	3.39	0.85
Fultz	2.65	1.02
Gladden	3.65	1.24
Kawvale	3.39	1.06
Leap	3.69	1.30
Purdue No. 1	2.43	1.02
Purplestraw	3.69	1.30
Rex	4.77	1.10
Rudy	3.51	0.82
Thorne	3.67	1.10
Triplet	4.00	1.10
Trumbull	3.71	0.85

kernel types. The Early Baart variety was found to have the highest thiamin content (6.90 μg per gram) among the hard wheat samples; and the Triplet variety was highest among the soft wheats (4.00 μg per gram). The thiamin values given in Tables I and II are in general agreement with those reported for the same varieties by Schultz, Atkin, and Frey (1941).

The hard wheats showed a range of 0.89 to 1.91 μg of riboflavin per gram, with an average value of 1.17. Among the soft wheats the range was 0.81 to 1.48 μg of riboflavin per gram with an average value of 1.07. No significant difference was found in the riboflavin content of hard and soft wheat. As shown in Tables I and II, the riboflavin content of wheat is considerably lower than that of thiamin. This is in confirmation of the result of a single bioassay reported by Morgan and Hunt (1935). However, the values reported in the present paper do not bear out the conclusion reached by Munsell and De Vaney (1933) that the riboflavin content of wheat varies directly with the thiamin content.

Riboflavin and Thiamin Values of Wheat Grown in Different Localities

Wheat samples obtained from the various wheat-producing areas of the United States were analyzed for thiamin and riboflavin. The values reported in Table III are for wheats of unknown variety but are representative of the localities from which they were obtained.

Although for certain areas only a few samples were examined, the general conclusion may be drawn that the locality from which the wheat is obtained has a direct bearing on its thiamin and riboflavin content.

This may in part be due to the kernel type (hard or soft) of the wheat grown in any particular area.

TABLE III

RIBOFLAVIN AND THIAMIN CONTENT OF WHEAT GROWN IN VARIOUS LOCALITIES

State	Number samples studied	Average thiamin	Average riboflavin
		μg/g	μg/g
Colorado	1	5.84	1.02
Idaho	1	3.91	0.89
Indiana	14	4.12	1.03
Kansas	7	5.01	1.14
Michigan	11	4.31	1.00
Montana	3	4.52	1.08
Nebraska	10	4.47	1.20
Ohio	3	3.65	1.10
Oklahoma	6	5.32	1.33
Oregon	2	4.66	0.95
Texas	9	3.91	1.42
Virginia	3	3.35	1.34
Washington	6	4.51	1.08

Correlation between Protein, Thiamin, and Riboflavin Content of Wheat

Table IV gives the thiamin and riboflavin content for wheats of different protein content. These values indicate a direct relationship between the protein content of wheat and its thiamin content. The wheat

TABLE IV

CORRELATION BETWEEN PROTEIN, THIAMIN, AND RIBOFLAVIN CONTENT OF WHEAT

Number samples studied	Range of protein content	Average thiamin content	Average riboflavin content
	%	μg/g	μg/g
5	7–9	3.93	1.02
14	10–12	4.05	1.24
15	13–15	4.78	1.10
15	16–19	5.03	1.24

samples having the highest protein content also contained the largest amounts of thiamin. No such direct relationship was found with riboflavin.

Influence of Environmental Conditions on Riboflavin and Thiamin Content of Wheat

Through the kindness of Mr. Karl F. Finney of the Hard Winter Wheat Quality Control Laboratory at Manhattan, Kansas, who supplied

the samples, a study was made of the riboflavin and thiamin content of varieties of wheat grown from the same seed but under different environmental conditions. The samples were grown by agricultural experiment stations and agencies cooperating in the winter wheat program. For a detailed description of the varieties and the conditions under which they were grown, the reader is referred to the bulletin, *Comparison of Winter Wheat Varieties Grown in Cooperative Plot and Nursery Experiments in the Hard Winter Wheat Region in 1939*, published in January, 1940, by the Bureau of Plant Industry of the U. S. Department of Agriculture.

TABLE V

THIAMIN AND RIBOFLAVIN VALUES OF WHEAT AS INFLUENCED BY VARIETY AND LOCATION

Locality	Kharkof		Blackhull		Tenmarq		Kawvale × Tenmarq		Oro × Tenmarq		Chiefkan	
	Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin	Thiamin	Riboflavin
	μg/g		μg/g		μg/g		μg/g		μg/g		μg/g	
Akron, Colorado	—	—	—	—	5.83	1.02	—	—	—	—	—	—
Alliance, Nebraska	4.13	0.95	—	—	4.55	1.19	3.91	0.81	4.35	1.22	4.13	1.22
Chillicothe, Texas	—	—	4.24	1.49	4.13	1.14	3.81	1.31	4.67	1.10	2.97	1.19
Denton, Texas	—	—	3.39	1.99	3.60	1.36	3.08	2.03	3.71	1.19	—	—
Goodwell, Oklahoma	4.75	1.22	5.10	1.19	5.83	1.06	5.83	1.90	5.93	1.69	4.45	1.19
North Platte, Nebraska	—	—	—	—	4.99	1.39	—	—	4.24	1.02	—	—

The results in Table V indicate that environmental conditions during the period of growth affect both the riboflavin and thiamin content of wheat, although Harris (1934), Leong (1939), and Scheunert and Schieblich (1936), using biological methods of assay, have reported that different soil fertilizer treatments were without effect on the thiamin content. It would seem desirable to carry out further studies (utilizing the more rapid chemical procedures) concerning the influence of climate, soil, etc., on the thiamin and riboflavin content of wheat.

Riboflavin and Thiamin Contents of White and Yellow Corn

Determinations of thiamin and riboflavin were made on samples of white and yellow corn obtained from the principal corn-producing areas of the United States. The results are shown in Table VI.

White corn was found to have a slightly higher thiamin content than yellow corn, a difference which was also observed by Akroyd and Roscoe (1929), although Schultz, Atkin, and Frey (1941) found no significant difference. No important difference was found in the riboflavin contents

of the two types. The average thiamin content of all the samples analyzed is somewhat lower than the value of 5.34 μg per gram reported by Schultz, Atkin, and Frey (1941).

TABLE VI

RIBOFLAVIN AND THIAMIN CONTENT OF WHITE AND YELLOW CORN

Kernel type	Number samples	Thiamin		Riboflavin	
		Range	Average	Range	Average
		μg/g	μg/g	μg/g	μg/g
White	12	2.54–7.40	4.40	0.92–2.29	1.32
Yellow	16	2.33–5.93	3.80	0.84–2.02	1.30

Riboflavin and Thiamin Contents of Various Wheat and Corn Products

Various wheat and corn mill products were analyzed for riboflavin and thiamin with the results given in Table VII. These values indicate that wheat germ is richer than corn germ in thiamin. However, the thiamin contents of the bran portions of these cereals were approximately the same. The riboflavin values of wheat and corn germ were considerably lower than the corresponding thiamin values.

TABLE VII

RIBOFLAVIN AND THIAMIN CONTENT OF VARIOUS WHEAT AND CORN PRODUCTS

Material	Number samples	Thiamin range	Riboflavin range
		μg/g	μg/g
Wheat germ	3	30–45	4–5
Corn germ	2	19–33	2–5
Wheat bran	3	5–6	3–4
Corn bran	1	4–5	1–2
Corn oil cake	1	9–10	6–7
Corn grits	5	0.5–1.0	0.3–0.5
White corn meal	3	1–2	0.7–0.8
Yellow corn meal	3	1–2	0.5–1.0

Summary

A study has been made of the thiamin and riboflavin content of wheat and corn, with the following results:

The hard-kernel types of wheat have been found to contain a higher thiamin content than the soft types but, with respect to riboflavin, there was little difference between the two.

In both wheat and corn there was a higher concentration of thiamin than of riboflavin.

The thiamin content of wheat appeared to be dependent on variety, protein content, and the environmental conditions under which it was grown. These factors appeared to be less important in the case of the riboflavin.

The thiamin value of white corn was found to be slightly higher than that of yellow corn, but little difference was found between the two kinds in respect to riboflavin.

Wheat germ had a higher thiamin content than corn germ, but the riboflavin value was approximately the same.

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THE DISTRIBUTION OF VITAMIN E IN PRODUCTS OF CEREAL MILLING[1]

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Vitamin E is of particular interest to the cereal chemist since some of the richer sources are the oils contained in the embryo of cereal grains. Of these wheat germ oil is the richest known source. Although our knowledge of this vitamin dates back less than twenty years, an extensive literature on the subject has been ably reviewed by Mattill (1939), Bacharach and Drummond (1939), and by Merck & Co. (1940).

The existence of a dietary factor essential to normal reproduction in rats was discovered independently by Evans and Bishop (1922), Mattill (1922), and Sure (1924). Wheat germ oil was found to be a potent source of this substance by Evans and Burr (1925), who designated it as the "anti-sterility vitamin fat soluble E." Its occurrence in other vegetable oils such as rice germ, cottonseed, and lettuce was later established, but animal products such as butter and eggs were found to contain only traces. The vitamin was isolated by Evans, Emerson, and Emerson (1936) from the nonsaponifiable fraction of wheat germ oil, in the form of two alcohols which were designated alpha- and beta-tocopherol respectively from "tokos" (childbirth) "pheros" (to bear) and the ending "ol" indicating an alcohol. Alpha-tocopherol was found to possess the formula $C_{29}H_{50}O_2$. Later these same workers isolated from cottonseed oil a third substance possessing vitamin E activity which they named gamma-tocopherol (Emerson, Emerson, and Evans, 1936).

Alpha-tocopherol was synthesized in 1938 by Karrer *et al.* (1938), Bergel *et al.* (1938), and Smith *et al.* (1938). It differs from the natural compound only in being optically inactive. The three tocopherols are closely related chemically, the beta and gamma forms being isomeric and differing from the alpha only in containing one less methyl group in the aromatic nucleus. No standard unitage such as exists for the other vitamins has as yet been satisfactorily worked out for E, and the potency of such preparations is therefore expressed in terms of the amount required to restore normal reproductive efficiency in E-deficient rats. The biological potencies of alpha-, beta-, and gamma-tocopherols are not identical and, while conflicting statements are to be found in the literature, the generally accepted activities are 1 to 3 mg for the alpha, about 5 mg for the beta, and 7 mg for the gamma form.

[1] Paper No. 34, Journal Series, General Mills, Inc., Research Laboratories.

Isolation of the naturally occurring tocopherols is accomplished by first preparing the nonsaponifiable matter, removing the bulk of the sterols, and further concentrating by partition between solvents, chromatographic adsorption, or high vacuum distillation. Final isolation and purification are carried out by forming derivatives such as the allophanates, half-succinates, or higher fatty acid esters.

The tocopherols are pale yellow, viscous oils which can be oxidized to tocoquinones of greatly lessened biological activity; solutions in fatty oils, however, are relatively stable to atmospheric oxidation. They possess marked antioxidant activity which has been found to be in inverse relation to biological potency.

Their physiological properties are primarily related to the reproductive functions. In the female rat, lack of vitamin E results in failure of the embryo to develop and subsequent resorption. In the male rat, continued deprivation results in testicular degeneration and complete and permanent sterility. Studies with larger animals and humans have shown very conflicting results and their requirements for this vitamin are still an open question.

In this study, our problem was concerned primarily with estimation of the low concentrations of tocopherol present in oils extracted from products of cereal milling. The animal assay procedure developed by Evans and Burr (1927) and by Palmer (1937) is inapplicable to the determination of the vitamin in such low concentrations. This difficulty may be overcome by feeding a concentrated preparation such as a nonsaponifiable but two inherent objections to the biological method still remain: first, only estimates of *relative* potency can be secured and no inference can be drawn regarding the kind of tocopherol present, and second, extensive preliminary assays are necessary in order to set the proper bioassay level.

These difficulties could be obviated if a satisfactory chemical test were available and, within recent years, several methods for the analytical determination of tocopherol have been proposed. These include the spectrographic procedure used by Drummond and co-workers, but applicable only to relatively pure material; oxidation to the tocoquinone either by gold chloride in a potentiometric titration as described by Karrer *et al.* (1938a); with ferric chloride in the original colorimetric iron-dipyridyl method of Emmerie and Engel (1938), or as modified by Parker and McFarlane (1940); and oxidation to the red-orthoquinone with nitric acid as developed by Furter and Meyer (1939).

Extensive investigation in our laboratory indicated that none of these chemical methods was applicable to the problem because of the presence of large amounts of interfering substances. In the case of the oxidation procedures of Karrer and Emmerie and Engel, the interfering materials

are reducing in nature and cause high results. Efforts to free the sample of these nontocopherol reducing compounds have not been successful. In the Furter and Meyer method, chromogenic substances are present which yield yellow and brown pigments upon oxidation with nitric acid and thus mask the red color of the orthoquinone. This latter method was selected as a basis for working out a suitable analytical procedure.

The method finally developed is based upon chromatographic fractionation of the nitric acid oxidation products upon activated alumina and is effective in separating the red orthoquinone oxidation product of tocopherol from the interfering pigments. This method will be described in detail elsewhere. It has been checked by extensive comparisons with the bioassay procedure and in the instance of preparations derived from wheat, which are known to contain alpha-tocopherol substantially, excellent agreement between the two methods has been found. It should be pointed out that no chemical methods yet developed are capable of distinguishing between alpha-, beta-, and gamma-tocopherols, and since the biological activities of these compounds differ widely it is necessary to resort to animal assay coupled with the results of chemical analysis to ascertain if a single tocopherol or a mixture is present.

In conducting such combined chemical and biological tests it is necessary to work with the nonsaponifiable matter rather than the oil itself, since only in this manner is it possible to secure an adequate concentration of the vitamin. In addition, the presence of large amounts of glycerides interferes with adsorption in the chemical procedure. Since the tocopherols are quite sensitive to oxidation in the presence of alkali and soaps, it is necessary to carry out these initial steps of saponification and extraction of the nonsaponifiable in such a manner as to permit of quantitative recovery of the vitamin in an unaltered condition. That this is accomplished by the technique employed is illustrated by the bioassay data in Table I.

TABLE I

COMPARISON OF BIOASSAYS OF WHEAT GERM OIL AND THE NONSAPONIFIABLE FRACTION DERIVED THEREFROM

Sample	Level fed	Biological activity	
		Total litter	Implant
		%	%
Wheat germ oil	500 mg	100	76
Nonsaponifiable =	to 500 mg of oil	100	86

In applying the method to the examination of mill products, large samples were extracted in modified glass Soxhlet extractors capable of dealing with three to five kilograms at a charge. Sufficient quantities of oil were thus extracted to enable both chemical and biological determina-

tions to be carried out upon the nonsaponifiables which were further concentrated by removal of a large part of the sterols present by crystallization from methanol.

The analytical results obtained with a series of hard wheat mill streams and products of milling are detailed in Table II and a comparison with bioassay values in Table III.

TABLE II

MILL YIELD, OIL CONTENT, AND TOCOPHEROL CONTENT AND DISTRIBUTION IN HARD-WHEAT MILLED PRODUCTS

Sample	Mill yield	Oil	Tocopherol in 100 g		
			In oil	Mill product	Distribution
	%	%	%	*mg*	*% of total*
Patent flour	60.3	0.83	0.003	0.03	2.0
First clear flour	9.4	1.78	0.082	1.46	17.4
Second clear flour	4.1	4.16	0.069	2.87	14.8
Red dog	2.7	5.83	0.099	5.77	20.0
Shorts	9.3	4.41	0.072	3.18	37.6
Bran	14.0	2.97	0.012	0.30	6.3
Germ	0.1	8.90	0.178	15.84	1.9
Whole wheat	—	1.54	0.059	0.91	—

TABLE III

TOCOPHEROL CONTENT AND BIOASSAY DATA UPON NONSAPONIFIABLE FRACTIONS

(Hard-wheat milled products)

Source	Tocopherol in sterol-reduced nonsaponifiable	Level fed	Bioassay	
			Tocopherol in sample fed	Total litter
	%	*mg*	*mg*	%
Whole wheat oil	2.34	42.7	1.0	83
Patent flour oil	0.15	212.0	0.3	0
First clear flour oil	4.18	24.0	1.0	57
Second clear flour oil	4.80	20.6	1.0	100
Red dog oil	6.16	16.3	1.0	100
Shorts oil	2.80	35.9	1.0	86
Wheat bran oil	0.29	346.0	1.0	67
Wheat germ oil	12.10	7.3	0.9	100

In considering the results of this study, it must be emphasized that the milled products examined were obtained from a single large mill and may not be entirely representative. In this study, we were as much concerned with establishing the analytical procedure upon a sound basis as with securing data upon the tocopherol content of the various fractions. It is pertinent, therefore, to first consider the comparative data presented in Table III. In these animal assays the amount of non-

saponifiable fed was adjusted so as to contain 1 mg of tocopherol based upon the chemical data, since by the bioassay technique employed in this laboratory, a dosage of 1 mg of alpha-tocopherol results in a total litter efficiency of about 85%. On this basis the majority of the results are in good agreement and, with the possible exception of the first clear flour, lie well within the error of the normal animal assay for vitamin E. These results confirm the relative precision of the analytical method, particularly when it is realized that all the bioassay values fell within the critical region upon the first test, since *no* preliminary "level setting" assays were made. They also indicate that the tocopherol of whole wheat is substantially alpha.

Turning now to the data presented in Table II, it will be noted that the major portion of the tocopherol is contained in the red dog, shorts, and clear flours, the patent flour being practically free and the bran quite low. Together, these latter fractions, while constituting 75% of the mill yield, account for only 8%, at most, of the total tocopherol, whereas the clear flours, shorts, and red dog, which represent 25% of the mill yield, contain 90% of the total, and the germ, with only 0.1% yield, accounts for 2%.

Since the embryo represents approximately 2.5% of the total weight of the kernel, it is obvious that much of the tocopherol found in certain of the milled products is attributable to the presence of pulverized germ. Using the value of 0.91 mg per 100 g as the tocopherol content of whole wheat, calculation indicates that approximately 55% of the total tocopherol is derived from the embryo, leaving 45% unaccounted for. Since the patent flour and bran are very low (8% of the total) it is apparent that the remaining 37% must originate in the tissues from which the clear flours, red dog, and shorts are derived and we thus secure the picture that vitamin E is concentrated in two regions of the wheat kernel, one being the embryo and the other the layers of endosperm closely adjacent to the bran. It must be emphasized again, however, that these results refer only to the products of one mill and the conclusions regarding distribution within the kernel require confirmation by more extended investigation upon a range of samples. It is possible, however, to state fairly definitely that not all the vitamin E of wheat is contained in the embryo, and also that this "nongerm" tocopherol is substantially alpha.

A study similar to the one described above has been conducted with the commercial milled products of durum wheat, the results being detailed in Tables IV and V.

The bioassay data in Table V also confirm the precision of the analytical results, although the agreement between the two sets of data is not quite as good as was the case with the hard-wheat fractions. The values

TABLE IV

MILL YIELDS, OIL CONTENT, AND TOCOPHEROL CONTENT AND DISTRIBUTION IN DURUM-WHEAT MILLED PRODUCTS

Sample	Mill yield	Oil	Tocopherol in 100 g		
			In oil	Mill product	Distribution
	%	%	%	*mg*	*% of total*
Semolina	53.7	0.75	0.034	0.255	16.5
First clear flour	4.3	1.52	0.034	0.517	2.6
Second clear flour	12.2	2.37	0.042	0.995	14.6
Red dog	10.1	4.55	0.038	1.729	21.1
Shorts	11.6	4.85	0.048	2.328	32.5
Bran	8.0	4.41	0.030	1.323	12.8
Durum wheat	—	2.07	0.052	1.076	—

TABLE V

TOCOPHEROL CONTENT AND BIOASSAY DATA UPON NONSAPONIFIABLE FRACTIONS

(Durum-wheat milled products)

Source	Tocopherol in sterol-reduced nonsaponifiable	Level fed	Bioassay	
			Tocopherol in sample fed	Total litter
	%	*mg*	*mg*	%
Whole wheat oil	2.33	42.8	1.0	57
Semolina oil	1.71	58.2	1.0	50
First clear oil	1.96	51.0	1.0	57
Second clear oil	2.49	40.0	1.0	100
Red dog oil	2.71	36.8	1.0	86
Shorts oil	2.00	50.0	1.0	71
Bran oil	1.30	77.0	1.0	57

of approximately 55% total litter efficiency found for four of the samples might be taken to indicate the presence of a tocopherol showing slightly less activity than pure alpha. Such a possibility is partly substantiated by the fact that the three samples showing the equivalent of full alpha activity are the second-clear, red-dog, and shorts oils in which the majority of the germ tocopherol would be concentrated. This would suggest that the nongerm tocopherol of durum wheat contains either a considerable proportion of beta- or a small content of gamma-tocopherol. It is impossible, however, upon the evidence available to make any definite statement on this point until more extensive data have been secured; the most that can be claimed with any degree of certainty is that the vitamin E of this particular wheat possesses a high biological activity approaching that of alpha-tocopherol.

The data given in Table IV indicate that the hard and durum wheats tested contain approximately equal amounts of vitamin E, but that the

distribution is somewhat different. Since the milling systems for the two wheats are dissimilar, no direct comparisons can be made. The semolina and patent flour fractions, however, should be roughly comparable as they both represent a close approximation to pure endosperm. It is therefore of interest to note that the semolina contains an appreciable quantity of the vitamin, amounting to 16.5% of the total, whereas patent flour is practically free. It does not appear probable that this sizable tocopherol content of semolina is due to germ contamination, since the oil contents of semolina and patent flour are practically identical.

In the durum milling system, no germ is secured and the tocopherol derived from the embryo must, therefore, be completely distributed over some of the other milled products. It is generally believed that the majority finds its way into the shorts, but the results obtained in this study suggest that a considerable amount goes into the bran, since this fraction is relatively high in both oil and tocopherol.

As with the hard wheat, computation indicates that the embryo cannot account for more than 50% to 60% of the total tocopherol found in the wheat. The principal difference between the two types of wheat appears to reside in the distribution of this "nongerm" tocopherol. In durum it seems to be fairly uniformly distributed throughout the endosperm, whereas in hard wheat it is localized in the layers closely adjacent to the bran.

It should be again pointed out that these results with durum wheat are representative of the mix and procedure employed in a particular mill and must, therefore, be regarded as indicative only until confirmed or disproved by more extensive studies.

Summary

The historical background, isolation, and chemical and physiological characteristics of the tocopherols are briefly reviewed and discussed.

Difficulties involved in the biological assay of materials containing low concentrations of the vitamin are described and the unsuitability of existing analytical procedures pointed out.

Reference is made to and a brief outline given of a method developed for the chemical analysis of such products as the oils extracted from milling products of cereal grains. This method involves chromatographic separation of the nitric acid oxidation products upon activated alumina.

Biological assay data are presented which indicate that the preparatory steps employed do not result in measurable loss of tocopherol and also that the results obtained by the chemical method correlate well with those secured by animal assay.

Analyses of milled products derived from hard and durum wheats are presented. These indicate that both wheats contain essentially similar amounts of vitamin E and suggest that this is present substantially as alpha-tocopherol. The distribution within the kernel differs, however. In both wheats, about 55% is located in the embryo and in the hard wheat the remainder is found in the endosperm layers closely adjacent to the bran, this latter tissue together with the bulk of the endosperm constituting patent flour being practically free from the vitamin. In durum wheat, the nongerm tocopherol is fairly uniformly distributed throughout the endosperm, the semolina containing appreciable amounts.

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THE APPLICATION OF THE THIOCHROME METHOD TO THE THIAMIN ANALYSIS OF CEREALS AND CEREAL PRODUCTS [1]

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For a period of more than forty years since the pioneer work of Eijkman in 1897 the vitamin B_1 content of cereals and cereal products has been of considerable interest to an ever-increasing number of investigators. From the early observations that whole rice grain would prevent the beriberi that resulted from dietaries largely comprised of polished rice came a recognition that cereals constitute an important class of foods aside from their content of starch, protein, and minerals. It is now generally agreed that cereal grains are one of the most important dietary sources of vitamin B_1 or thiamin.

The early work on this subject was necessarily slow, partly because of the absence of adequate analytical methods. The use of fowls and later rats as subjects for assay left much to be desired. These methods were expensive and time-consuming and available only to a limited number of specially equipped laboratories.

Today the picture has been decidedly changed by the development of microbiological and chemical procedures. No longer is it necessary to make a considerable investment of money for a vitamin B_1 analysis that may be completed a month or two after the sample has been submitted for assay. Results can now be obtained within a few hours and in some instances within a few minutes at a modest cost.

One of these rapid methods is the thiochrome procedure. In 1935 Peters discovered that the oxidation of thiamin resulted in its conversion

[1] Paper No. 32, Journal Series, General Mills, Inc., Research Laboratories.

to a strongly blue fluorescent substance which was soon identified and named thiochrome. In the following year Jansen (1936) utilized this property of the vitamin as the basis of an analytical method. The vitamin was oxidized, extracted with isobutanol, and the fluorescence of the extract compared to that of a standard quinine solution. Two years later Kinnersley and Peters (1938) reported that this method might in some instances fail to record the full vitamin activity. They found that the cause was the presence of cocarboxylase, the pyrophosphate ester of thiamin. This substance possesses the equivalent physiological potency of the vitamin but the thiochrome oxidation product is insoluble in isobutanol and thus escapes detection. This difficulty was overcome by Hennessy and Cerecedo (1939) by enzymatically converting cocarboxylase to thiamin during the extraction of the sample. These investigators also introduced the use of zeolite as an efficient and convenient means of separating the vitamin from impurities which interfere in its determination.

For the past two and one-half years the thiochrome method has been employed in this laboratory for evaluating the thiamin content of cereals and cereal products. During this period a large number of samples have been examined and minor modifications of the method have been introduced for greater operating convenience. Some of the observations made during these investigations are being reported here.

Comparison of Thiochrome and Bioassay Methods

Hennessy and Cerecedo (1939) have compared the results obtained from their modification of the thiochrome method with those obtained by bioassay. Their list includes several samples of cereals and in all cases excellent agreement was obtained by the two types of methods. These comparisons have been extended by including a wider variety of cereal products and determining the thiamin content by both the thiochrome and rat-growth methods. The assay values are given in Table I.

In most instances the results from the two methods are in very satisfactory agreement and in no single sample is the discrepancy sufficiently great to throw serious doubt on the validity of the thiochrome values. The bioassay method is susceptible to an experimental error which somewhat limits its value as a comparison standard. In the list (Table I) some of the bioassays were made without any knowledge of the thiamin content. Accordingly, the levels chosen for the assay required guesswork. This accounts for some of the qualifying statements about the actual rat-growth values. In other cases the samples were first analyzed by the thiochrome method. Bioassay levels chosen from these values have never failed to give growths comparable to those from the thiamin standard.

TABLE I

COMPARISON OF THIAMIN VALUES OBTAINED BY RAT GROWTH AND THIOCHROME METHODS

Sample	Thiamin in micrograms per gram	
	By rat growth	By thiochrome
	μg per gram	*μg per gram*
Wheat germ	30	31.5
Red dog flour	Slightly less than 15	15.1
Fortified cereal I	Slightly less than 10.5	9.7
Wheat cereal I	More than 7.5	7.9
Wheat cereal II	6	6.3
Fortified cereal II	Less than 6.6	6.1
Long extraction flour I	6	6.0
Wheat cereal III	6	5.8
Oat meal	4.8	5.4
Fortified flour	Slightly less than 5.2	5.4
Whole wheat flour	Between 4.5 and 5.4	5.1
Long extraction flour II	Less than 2.4	1.8

Discussion of the Thiochrome Method

The amount of sample taken for the assay is determined largely by its thiamin content. It is preferable to use a quantity which will contain approximately 9 μg or 3 International Units. This quantity has been chosen in order to have about 1 μg in the 5 ml of purified extract taken for oxidation; and also be similar in this respect to the standard thiamin solution which is simultaneously carried through the whole procedure. This use of the standard solution is desirable since its analysis serves as a check on the various operations and counteracts the effect of small errors arising from such steps as filtration and the base exchange in the zeolite purification.

In the analysis of low-potency materials such as patent flour a compromise must be made since it is difficult to extract the desired quantity. Four to five grams is used with 50 ml of the solvent and a larger aliquot is passed through the zeolite to bring the final concentration as near as conveniently possible to the desired 1 μg per 5 ml.

The use of a quantity of sample which will supply 9 μg represents the ideal. For practical purposes a considerable variation from this figure will give satisfactory results. As Hennessy and Cerecedo (1939) have pointed out, the base exchange is efficient over a considerable range. If the sample contains between 5 and 15 μg of thiamin, satisfactory results can be obtained without changing the amounts of solutions used in the zeolite purification.

For the extraction the originally proposed acetic acid solvent has been used. Other acids will serve satisfactorily, since their use is pri-

marily designed to stabilize the thiamin during the extraction.[2] If mineral acids are employed, subsequent partial neutralization must be effected with a buffer salt; with acetic acid, caustic will produce the desired buffer action.

Figure 1 is a photograph of the extraction equipment employed. It is merely a steam heated water bath designed for six simultaneous ex-

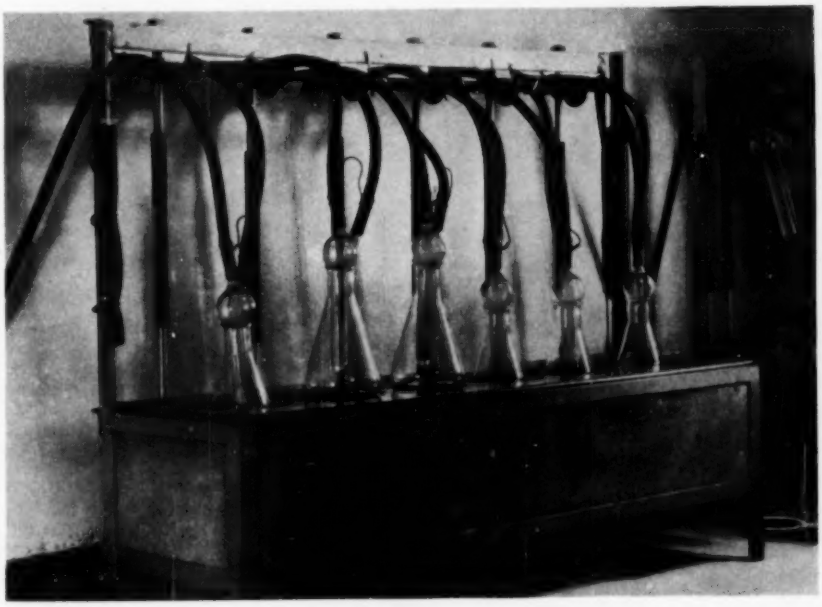

Fig. 1. Extraction apparatus.

tractions. The extraction vessel is a 250-cc Erlenmeyer flask and the condensers are of the "cold finger" type. They possess the advantage of being inexpensive and easy to use. They require no ground joints or stoppers which tend to stick or contaminate the extract. Figure 2 is a sketch showing the condenser construction. Cold water flows through the interior to keep the outer condensing surface cooled.

The next step involves enzymatic hydrolysis with a preparation of diastase. Either the originally proposed takadiastase or the preparation known as Clarase is satisfactory (Proceedings of Vitamin Assay Conference, 1940). The enzymatic hydrolysis has two functions: first, it breaks down the starch, facilitating extraction of the thiamin and subsequent clarification of the extract; second, it converts any cocarboxylase

[2] Recent observations suggest that more drastic extraction conditions may be required in the analysis of bread. One sample which was extracted by autoclaving with 0.1*N* H_2SO_4 for 20 minutes at 15 lbs pressure gave a 10% higher value for thiamin than when extraction was carried out by refluxing with acetic acid. Other samples of bread, however, have given the same values by both extraction methods. Where autoclaving is used the sulfuric acid solvent is preferred to acetic acid.

into free thiamin. With most cereals and cereal products the latter action is not particularly important since the quantities of cocarboxylase present are negligibly small. It has been shown, however, that wheat germ is an exception and must be hydrolyzed before oxidation to the thiochrome. Bread is another and even more important exception. In

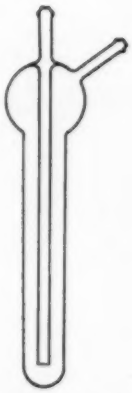

Fig. 2. "Cold finger" type of condenser.

some of the early analyses of this type of product serious discrepancies were observed. Low values and poor checks from replicate determinations were obtained. This difficulty was removed only after raising the temperature of the enzymatic hydrolysis or prolonging the digestion period considerably. It seemed probable that some reaction other than rapid starch hydrolysis was required for reliable analytical results. Since the hydrolysis of cocarboxylase is relatively slow the difficulties were attributed to the presence of this thiamin derivative. However, the flour from which the bread was prepared contained no cocarboxylase and it was necessary to assume that phosphorylation of the thiamin occurred during the yeast fermentation of the dough. A preliminary examination of this possibility was made by examining at various intervals of the thiamin content of a suspension of flour, thiamin, and yeast. Table II shows the results.

When hydrolysis with takadiastase was used for preparing the samples for assay there was no significant change in the thiamin during fermentation. When takadiastase was omitted, the amount of free thiamin decreased. After three hours nearly one-third of the original thiamin could not be measured without the use of the enzyme. While this experiment does not prove that thiamin is converted to cocarboxylase during fermentation it does demonstrate the importance of thorough enzymatic hydrolysis in the analysis of bread.

TABLE II

THE THIOCHROME ANALYSIS OF FLOUR-THIAMIN-YEAST SUSPENSIONS
(Thiamin in micrograms per gram of flour)

Fermentation time	Results from thiochrome analysis	
	With takadiastase	Without takadiastase
	μg per gram	*μg per gram*
2 minutes	4.98	4.89
30 minutes	4.80	3.72
1 hour	4.89	3.42
3 hours	4.62	3.24

The next steps involve clarification and base exchange. For clarification either centrifugation or filtration may be used. Centrifugation has the advantage of preventing the possible loss of thiamin from the extract by adsorption which may occur on filter paper. On the other hand, clarification is less complete and as a consequence the flow of the extract through the zeolite is retarded. While this is a minor item there is a tendency for the insoluble material to deposit on the zeolite particles and more rapidly lower the efficiency of the base exchange when the zeolite is used for a number of successive determinations. Filtration has been found to be satisfactory and convenient. A small loss of thiamin apparently takes place but this is entirely compensated for by treating the standard thiamin solution in the same manner. It is possible, however, that different types of filter paper may vary. They should be checked by comparing the thiamin contents of two aliquots of the standard, one of which has been filtered.

For the base exchange, tubes similar to those described by Hennessy [3] at the 1939 Convention of the American Association of Cereal Chemists have been used. Figure 3 shows a photograph of a series of these tubes ready for operation. One operator can easily handle 12 to 15 zeolite purifications simultaneously.

Keeping in mind that the base exchange step was introduced as a means of eliminating interfering impurities it was of interest to determine how the analyses of cereals would be affected if this operation was omitted from the procedure.

Several experiments have been carried out in which the filtered extract was oxidized directly. Table III compares the results obtained with and without the zeolite treatment.

In no single instance was there more than 5% difference between the values from the two methods. In nearly all the samples the agreement was as good as can be obtained from replicate assays by the regular thio-

[3] Unpublished.

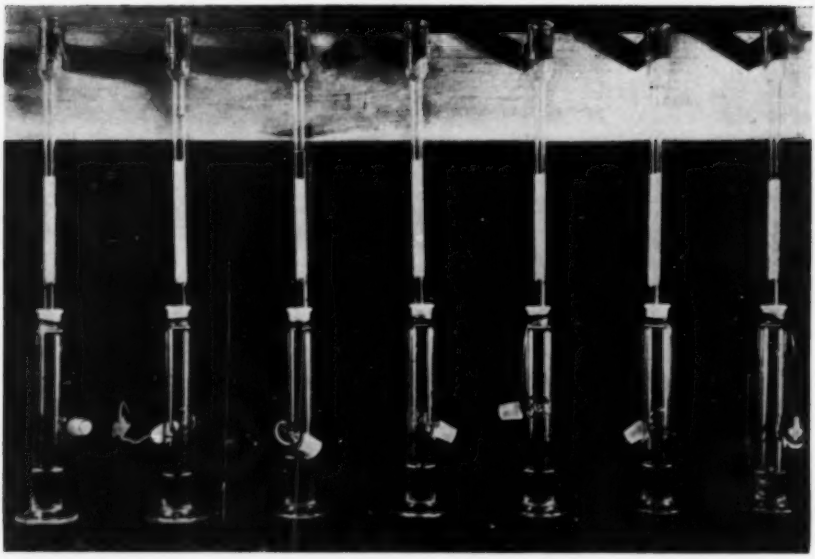

Fig. 3. The zeolite purification.

TABLE III

COMPARISON OF THE THIOCHROME VALUES OBTAINED WITH AND WITHOUT THE ZEOLITE PURIFICATION

Sample	Thiamin in micrograms per gram	
	With zeolite	Without zeolite
	μg per gram	*μg per gram*
Enriched flour	4.23	4.20
Patent flour	0.81	.84
Corn meal	1.74	1.71
Low grade flour	5.70	5.49
Cereal I	6.57	6.63
Cereal II	4.41	4.20

chrome procedure. At least in the samples presented in this table it is believed that the amounts of impurities which carry through the determination and affect the fluorescence readings are negligible. When the zeolite is omitted care must be taken that the isobutanol solution of the thiochrome is entirely clear. A greater tendency to cloudiness will be observed, but this can be overcome by using larger amounts of finely ground sodium sulfate followed by brief centrifuging.

To determine whether or not the method could be simplified further the extraction process has been examined. A few experiments have been tried by simply shaking the sample with a 25% potassium chloride

solution in dilute acetic acid, filtering, oxidizing, and measuring the fluorescence of the isobutanol extract. Table IV compares the results thus obtained with those given by the regular procedure.

TABLE IV

COMPARISON OF THE THIAMIN VALUES OBTAINED BY THE REGULAR THIOCHROME PROCEDURE AND BY OXIDATION OF KCl EXTRACTS

	Sample	Regular thiochrome method	Oxidation of KCl extracts	Difference
		μg per gram	*μg per gram*	%
1	Enriched flour	3.90	3.93	− 0.8
2	Patent flour	0.75	0.69	− 8.0
3	Low grade flour I	8.64	8.58	− 0.7
4	Whole wheat flour I	4.26	4.38	+ 2.8
5	Low grade flour II	5.70	6.00	+ 5.3
6	Low grade flour III	30.9	30.6	− 1.0
7	Whole wheat flour II	5.1	4.59	−10.0
8	Red dog	15.0	13.6	− 9.3
9	Rice bran	37.5	34.2	− 8.8
10	Rice polishings	30.0	27.6	− 8.0
11	Bread (air dried)	3.75	1.83	−51.0

In only one instance was there an outstanding difference between the results from the two methods. This was the sample of bread where the potassium chloride extract contained less than 50% of the amount obtained by the regular thiochrome method. Such a result would be expected in view of the necessity for enzymatic hydrolysis to liberate all the vitamin as free thiamin.

Of the first six samples of flour only the patent showed a material percentage difference. It is believed that this can be attributed to the very low thiamin content since the 8% difference observed between the two methods is also frequently observed in replicate analyses by the complete thiochrome method.

Samples 7, 8, 9, and 10 gave 8% to 10% lower values by direct oxidation of the potassium chloride extracts. Whether this difference can be removed by some modification of the extraction procedure, or whether diastasis is required to evaluate the total thiamin content, remains to be determined. In either event the relatively high efficiency of extraction by merely shaking with a salt solution offers considerable promise and is being further studied.

Especially in the case of enriched flours the use of this procedure should prove to be of material value. Not only is all the added thiamin extracted but also practically all of the vitamin naturally present in the flour. Assay results are in essential agreement with those obtained by the regular thiochrome method and since the complete analysis can be

done in a comparatively short time the procedure is well adapted for purposes of controlling products.

Experimental

The thiochrome method used in the present studies is that described by the American Association of Cereal Chemists (1941). For the analyses of flours by extraction with potassium chloride solution the following procedure was employed:

Two and one-half grams of "enriched" flour was placed in a 250-ml Erlenmeyer flask and carefully suspended in 50 ml of 25% potassium chloride solution in 2% acetic acid. The suspension was shaken intermittently over a period of 15 minutes, then filtered, and 5 ml of the filtrate was oxidized and its fluorescence determined as in the regular thiochrome procedure. A standard solution containing 10 μg of pure thiamin chloride in 50 ml of the 25% potassium chloride-2% acetic acid solution was similarly filtered and a 5-ml portion of the filtrate oxidized and its thiochrome content determined. "Blanks" were determined for the unknown and standard and these values deducted from the fluorescence readings of their corresponding oxidized solutions. The "corrected" value for the standard is the fluorescence of 1 μg of thiamin and by simple proportionation the value for the unknown was determined.

Example: The fluorescence of an isobutanol extract of 5 ml of the unknown solution was 27.5 divisions. The blank was 3. Fluorescence of the standard was 27.5 with a blank of 2.5. Thus,

$$27.5 - 3/27.5 - 2.5 \times 50/5 \times 1/2.5 = 3.92 \ \mu g/gram.$$

Summary

The thiamin contents of a variety of cereals have been determined by both rat growth and thiochrome assays. The results obtained by these two methods are in essential agreement. Operating details of the thiochrome procedure are discussed and abbreviated techniques applicable to certain types of cereal products are reported.

Acknowledgments

The authors wish to acknowledge their indebtedness to Mr. B. R. Homrich and Miss Claire Frederick for some of the animal assays reported in this paper. Other bioassays were made by Mr. H. J. Cannon of the Laboratory of Vitamin Technology, Chicago, Illinois.

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QUICK TEMPERING OF WHEAT FOR EXPERIMENTAL MILLING

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It has always been a disadvantage to wait overnight for wheat samples to temper before milling. This is particularly true during the harvest when grain buyers are especially anxious to learn as quickly as possible the milling and baking characteristics of new wheat samples.

For over a year we have used a surface-active agent in our tempering water whenever we have wanted rapid results. In the past we were forced to temper our hard spring and winter wheats from 8 to 18 hours or overnight in order to secure the best milling results. By use of a wetting agent we have been able to cut our tempering time from overnight to two to three hours.

Our procedure is to do a moisture test on the wheat, then clean and weigh 2,000 g of the sample. A table is made indicating the correct number of milliliters to be added to a given weight of wheat at any moisture content to bring the moisture level to 15.0%. The sample is shaken thoroughly with the added water in an air-tight, screw-top glass container and when tempered sufficiently long it is milled.

For quick tempering we merely substitute approximately 0.1% solution of Aerosol OT (sodium dioctyl sulfosuccinate) in place of the tap water ordinarily used for tempering. This wetting agent is made by the American Cyanamid and Chemical Corporation. No doubt there are many other surface-active agents which might give equally good results.

Some wheats which are particularly difficult to temper may require four to five hours for the best results. The optimum time for the

wheats handled by the laboratory in any given crop year can be determined with a few trial experiments, using a tempering time from two to five hours with the solution of the wetting agent as compared with the overnight procedure with water on the same wheat. If, for any reason, the sample tempered with the wetting agent has to stand for a longer period of time the results are entirely satisfactory.

With the amounts of the particular surface-active agent used, we have noticed no difference in the milling yield, ash, protein, mixing curve, or baking results of the flours as compared with the flours milled by the usual long tempering method. The saving of time with the resultant speed in obtaining milling and baking results is sometimes a great asset in purchasing wheat.

BOOK REVIEW

Cereal Laboratory Methods, Fourth Edition. Compiled by Committee on Revision, American Association of Cereal Chemists, 110 Experiment Station Hall, College of Agriculture, Lincoln, Nebraska, 1941. Price $2.50.

The fourth edition of *Cereal Laboratory Methods* bears abundant witness to the rapid strides that have been made in the field of cereal chemistry since the publication of the first compilation of methods nearly 20 years ago. Even the third edition, which seemed like a rather imposing volume when it was published in 1935, seems slender and inadequate in comparison with this new edition. In spite of the fact that the size of the present volume has been considerably reduced through the use of charts instead of tables for conversion of ash and protein to different moisture levels, the fourth edition is still half again as large as the 1935 volume.

Recent progress in the development of new methods and the improvement of older methods for testing the baking quality of various types of flour is especially evident. All these baking test methods have been rewritten and amplified in accordance with the recommendations of the various committees of the A. A. C. C. The methods as they now stand are more clearly presented than in the past, and useful information is included on the interpretation of results and the descriptive terms used in scoring the baked products. This new edition makes it more than ever apparent that the cereal chemist has developed distinctive methods for testing the materials that are produced and utilized in the plants in which he works. A large number of the methods given in this book are taken from the files of *Cereal Chemistry* and there is less dependence than heretofore upon methods from other sources.

Turning first to the new material, the book contains four new chapters dealing with Experimental Milling, Rye, Malt, and Experimental Macaroni Processing. Chapter II, on experimental milling, will be welcomed by many as it gives recommendations and specifications for the milling equipment used for both bread wheats and durum wheats, as well as flow sheets for experimental milling. This is probably as comprehensive and yet compact a treatise on experimental milling as has ever appeared. Chapter V, on rye, includes two methods for detection of ergot, six methods for the determination of rate of gelatinization, and methods for the determination of lactic, acetic, and butyric acids. Chapter VI, on malt, gives methods for physical and chemical testing of malt. Among the chemical methods are determinations of extract, color of wort, total wort nitrogen, diastatic power, alpha-amylase, and proteolytic activity. Chapter X, on experimental macaroni processing, gives directions for production of disks for color measurement, and outlines the process of manufacturing macaroni on experimental equipment. This is supplemented by new material in Chapter XI, dealing with the determination of color and cooking characteristics of alimentary pastes.

For the first time the terms thus far defined by the Committee on Definitions of Technical Terms have been included in a book of methods. These terms are given in Appendix A as a glossary of cereal chemical terms. An innovation which will be helpful to cereal chemists is the excellent treatise on statistical principles and experimental errors which constitutes Appendix B. This treatise will fill a long-felt need for a brief and easily understood discussion of the applications of statistical principles to the analysis of cereal chemical data. Among the statistics and procedures explained are variance, standard deviation, analysis of variance, the t test, and the correlation coefficient. Included in Appendix B is an interesting compilation of experimental errors in the chemical and physical determinations commonly employed in cereal laboratories. In each case the standard error of a single determination is given.

Appendix C, on preparation and standardization of solutions, will be appreciated by every cereal chemist. Instructions are given for the common acids and alkalies and a number of salt solutions and buffer solutions.

The charts for correcting ash and protein to either the 13.5% or 15.0% moisture basis are convenient and compact. These four charts take the place of 72 pages of tables. If these charts were to be sold separately they would undoubtedly find wide acceptance among cereal chemists.

These new chapters, appendices, and charts, however, are by no means the only changes in the book, as revisions and additions have been made throughout. To mention some of the more important changes, to Chapter III, on flour and semolina, have been added sections on the determination of thiamin by the thiochrome and

fermentation methods and the qualitative and quantitative determination of iron. The Snell and Strong method for riboflavin is recommended but not described.

Chapter IV, on feeds and feeding stuffs, has been supplemented with methods for solids in syrups and molasses, fat in dried milk products, lactose in mixed feed, and methods for determination of phosphorus, manganese, chlorine and iodine.

Additional methods for the determination of staleness in bread are given in Chapter IX. To Chapter XII has been added a simple method for the determination of the specific volume of creams, cake batters, and icings. Chapter XIII, on yeast foods and flour improvers, has a new section on bleaching agents made up of methods formerly included in the chapter on flour and semolina and embracing new qualitative and semiquantitative methods for benzoyl peroxide.

The tables at the end of the book have been amplified, making the cereal chemist less dependent upon his chemist's handbook.

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